YELLOW FEVER INSTITUTE, BULLETIN No. 13.
Treasury Department, Public Health and Marine-Hospital Service.
WALTER WYMAN, *Surgeon-General.*

REPORT

OF

WORKING PARTY No. 1,

YELLOW FEVER INSTITUTE.

A STUDY OF THE ETIOLOGY OF YELLOW FEVER,

BY

HERMAN B. PARKER, Assistant Surgeon.
GEORGE E. BEYER, Acting Assistant Surgeon.
O. L. POTHIER, Acting Assistant Surgeon.

MARCH, 1903.

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
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PREFACE.

ORGANIZATION OF WORKING PARTY NO. 1, YELLOW FEVER INSTITUTE.

TREASURY DEPARTMENT,
U. S. MARINE-HOSPITAL SERVICE,
Washington, March 5, 1902.

SIR: Referring to Department approval of September 25, 1901, of the formation within this service of a yellow-fever institute, I have the honor to invite your attention to as much of the plan of organization as says, "The object of the institute is to collect facts concerning yellow fever, to designate specific lines of inquiries, and to make them."

In accordance with this plan and in view of the fact that the etiology of yellow fever seems still to be involved in much doubt, it seems desirable to make further investigations into this subject. With this end in view an officer of the service, Asst. Surg. Herman B. Parker, has been receiving special training in the hygienic laboratory, and it is desired to associate with him Dr. George E. Beyer, professor of biology, Tulane University of Louisiana, and Dr. O. L. Pothier, pathologist to the Charity Hospital, New Orleans, La., who have both made a special study of the transference of disease by mosquitoes and have recently made a report to the Orleans Parish Medical Society (November 30, 1902) on this subject.

In view of the fact that yellow fever prevails to only a very limited extent at the present time, it is not deemed advisable to dispatch this working party immediately, but it is under contemplation to do so as soon as an outbreak of any magnitude occurs in either Mexico, Central or South America.

In order to prepare for this contingency, therefore, I have the honor to request authority, when the exigency arises, to nominate the above-named Drs. George E. Beyer and O. L. Pothier as temporary acting assistant surgeons, U. S. Marine-Hospital Service, for duty in making the above investigations.

This request is made necessary by the fact that Drs. Beyer and Pothier are engaged in the practice of medicine and in teaching in the

Tulane University in New Orleans, and will have to make preparations and arrangements for a prolonged absence from the scene of their present duties.

Respectfully, WALTER WYMAN,

Supervising Surgeon-General, M. H. S.

The SECRETARY OF THE TREASURY.

WASHINGTON, D. C., *March 6, 1902.*

Approved:

O. L. SPAULDING, *Acting Secretary.*

TREASURY DEPARTMENT,
U. S. MARINE-HOSPITAL SERVICE,
Washington, D. C., April 25, 1902.

SIR: You are hereby relieved from duty in the hygienic laboratory and appointed chairman of a working party of medical officers for the purpose of making scientific investigations of yellow fever, malarial fever, and dengue, and other diseases common to the United States and Mexico.

For this purpose you are directed to proceed to Vera Cruz, Mexico, where, in conjunction with Drs. Beyer and Pothier, constituting the other members of this working party, you will enter upon these investigations.

You will make a monthly report to the Bureau as to the progress of the working party, and upon the completion of the investigations will make full report of the same.

Respectfully, WALTER WYMAN,

Surgeon-General, M. H. S.

Asst. Surg. H. B. PARKER,
Hygienic Laboratory, Washington, D. C.
(Through Director.)

YELLOW FEVER INSTITUTE.

Treasury Department, Bureau of Public Health and Marine-Hospital Service,

WALTER WYMAN, Surgeon-General.

BULLETIN No. 13.

Section B.—ETIOLOGY. P. A. Surg. M. J. ROSENAU, Chairman of Section.

A STUDY OF THE ETIOLOGY OF YELLOW FEVER,

By HERMAN B. PARKER, *Assistant Surgeon*,
GEORGE E. BEYER, *Acting Assistant Surgeon*,
O. L. POTHIER, *Acting Assistant Surgeon*.

1. LETTER OF TRANSMITTAL WITH CONCLUSIONS.

TREASURY DEPARTMENT,
BUREAU OF
PUBLIC HEALTH AND MARINE-HOSPITAL SERVICE,
New Orleans, La., February, 17, 1903.

SIR: In compliance with the Bureau orders of April 25, 1902, wherein a working party consisting of Asst. Surg. Herman B. Parker, and Acting Asst. Surgs. George E. Beyer and O. L. Pothier, working in conjunction with the yellow fever institute, were to proceed to Vera Cruz, Mexico, and begin the investigation of yellow fever and diseases common to Mexico and the United States, we have the honor to state that the working party arrived in Vera Cruz on May 12, 1902, secured quarters for the laboratory and began these investigations. This work terminated about the end of October. Since then we have been studying the material brought to this country and collaborating the notes made in Mexico. This work is concluded and we have the honor to present the report of the investigation.

We have to acknowledge the assistance and encouragement from every officer of the Mexican Government with whom we came in contact, whether directly or indirectly connected with the department of medicine. We especially acknowledge the courtesies extended by

Governor Dehesa and his staff of the state of Vera Cruz and by Dr. Eduardo Licéaga, president of the superior board of health of Mexico. Through them every channel was opened for the proper study of the disease.

To our daily companions, Drs. Matienzo, del Rio, and Iglezias, a commission appointed by the Mexican Government to associate with and assist us with their valuable clinical experience, we are indebted for suggestions and their uniform interest shown in the work.

In the proper study and classification of this new parasite, the working party desires to express its thanks to Mr. J. C. Smith, of New Orleans, La., for valuable aid and suggestions in working out the life history of the organism.

The United States Consul, Mr. W. W. Canada, and Acting Asst. Surg. S. H. Hodgson, United States Public Health and Marine-Hospital Service, were untiring in their efforts to make this work a success and to them we are indebted for much assistance. To Mr. Alexander M. Gaw many thanks are due for his interest in procuring and selecting the experimental material.

Following are our conclusions: 1. That the bacteriologic examination of the blood of cases of yellow fever during life and the blood and organs at autopsy performed immediately after death in uncomplicated cases is negative.

2. That *Stegomyia fasciata*, when contaminated by feeding on a case of yellow fever forty-one and a-half hours after the onset of the disease and subsequently fed on sugar and water for twenty-two days, one and a-half hours, can, when permitted to feed on a nonimmune individual, produce a severe attack of the disease.

3. That *Stegomyia fasciata*, contaminated by feeding on a case of yellow fever and after varying periods killed, sectioned, and appropriately stained, presents with regularity a protozoan parasite, the *Myxococcidium stegomyiae*, that can be traced through a cycle of development from the gamete to the sporozoite.

4. That *Stegomyia fasciata* fed on blood from a case of malarial fever, on normal blood, or artificially fed, does not harbor the parasite indicated in conclusion 3.

Respectfully,

HERMAN B. PARKER,
Assistant Surgeon,
GEORGE E. BEYER,
Acting Assistant Surgeon,
O. L. POTHIER, M. D.,
Acting Assistant Surgeon.

The SURGEON-GENERAL,
U. S. Public Health and Marine-Hospital Service,
Washington, D. C.,
Chairman Yellow Fever Institute.

2. PRELIMINARY.

A.—THE OBJECT OF THE WORKING PARTY.

It is almost unnecessary to review the influence that yellow fever now exerts over the economics of that part of the United States that borders on the South Atlantic and Gulf seaboards. In past years the experience was that in certain seasons of the year no city on the whole Atlantic seaboard was exempt from yellow fever, as the great epidemics of Boston, New York, Philadelphia, Baltimore, and Charleston testify. With the advancement of sanitary science, however, the disease crept slowly southward until it is now outside the confines of the United States. There now remains what is called "infectible territory;" that is, territory which, if the disease is introduced, furnishes the medium for the propagation of the disease. According to the quarantine laws, this territory includes all of the United States south of the southern border of the State of Maryland. In the light of recent investigations, it includes all that territory where the mosquito, the *Stegomyia fasciata*, finds its natural breeding place. The cities and towns in the United States where the insect thrives have not been accurately tabulated; from an epidemiological point, it would not be necessary or wise to make such a classification, except as a matter of interest, of more importance is it to study those towns that furnish the natural breeding places for this insect, for, should it be absent for the time being, there is always the danger of introduction through commercial channels from one of the centers of distribution, and establishing a habitat in a season or a part of a season.

Broadly speaking, that which constitutes a natural breeding place for this insect is the house, town, or city that stores its water supply in small unprotected containers for daily consumption during the period of flight, i. e., from March to November on our Gulf coast. These conditions supplied, there only awaits the time when commerce, either ships or railways, introduce the insect for domestication.

In what part of the United States are these conditions supplied? Beginning with Charleston, on the Atlantic coast, to the Mexican border, on the Gulf, and on the lines of railway and water communication in the interior from the centers of distribution.

All the cities and towns included in the area of infectible territory are not equally incriminated. Charleston, Savannah, and Brunswick now harbor few, if any, *Stegomyia fasciata*. Mobile, with its good

water supply, is rapidly eliminating not only the chances but the danger of infection; Jacksonville, Miami, Key West, Tampa, New Orleans, Brownsville, Eagle Pass, and Galveston now harbor *Stegomyia fasciata* in abundance.

Owing to the character of the water supply or system of the above-mentioned cities they furnish the same natural breeding places for this insect as Cuba, Mexico, Central and South American ports, where yellow fever prevails all the year. Owing, however, to periods of cold during the winter months, the spring and early summer generations are usually not numerous. From the end of August to the first of November they will be found in the same numbers as in the Tropics. There are, however, seasonal prevalences when the insect prevails in great numbers in the early spring months, the same as in more tropical regions.

It must not be forgotten that the species *Stegomyia fasciata* is not a native mosquito in our own country, but is domesticated more particularly in the places mentioned through the medium of commercial intercourse. This fact can not be disputed when the habits and breeding places are taken into consideration, the breeding places not being furnished until the beginning of civilization on this continent.

As a result of the existence of an area of infectible territory, and the prevalence of the disease in neighboring islands and mainland, a quarantine was established whereby ships and passengers from suspicious or infected territory were detained under observation after disinfection for periods varying from five to forty days or longer from the infected place. In recent years the maximum period of incubation of the disease, five days, was selected as the detention period; this continues in force at the present time.

Prior to the year 1900 disinfection for yellow fever approached, as nearly as chemical disinfection would accomplish it, the sterilization of every part of the ship. Steam, formaldehyde, and sulphur were used energetically to accomplish this purpose. This process was in accord with the knowledge of the times and undoubtedly limited the infection in numbers of cases. It did not meet the requirements, however, and infection from time to time was introduced and produced epidemics with varying severity without serious reflection on the quarantine officer, through whom it passed, or his methods of applying the disinfection. When one takes into consideration that the quarantine officer knew nothing of the etiology of the disease which he was striving to prevent, the wonder is that accidental infections were not more numerous than they were. In other words, the same methods were employed as in dealing with plague, cholera, or other quarantinable disease, caused by vegetable organisms, when there should have been employed an efficient insecticide, directed to the destruction of the mosquito.

The statement that disinfection of ships should have been superseded by the destruction of mosquitoes needs a definite explanation. The whole mass of corroborative evidence is that the *Stegomyia fasciata* is the medium for the transference of the disease. This is looked upon as a definite scientific fact. Is it, or is it not, the only means of transference? To prove a negative assertion conditions must be supplied to produce the disease at will or under constant conditions. In the whole history of the disease such data, to this date, are wanting. When one instance occurs and can be repeated the new factor can then be taken into consideration.

Knowing, then, as we do, that an individual sick with the disease harbors the infection in his blood, and that a mosquito which has bitten such a case can, after a period, carry the disease to a susceptible individual through the act of feeding, there remains to be found the cause or organism that is the infecting agent.

What advantage is to be derived by knowing the cause of the disease? There is nothing of more importance to the commerce of the whole United States, and especially our Southern States, than to say definitely that the cause of the disease is, or is not, a *protozoan* parasite, and that there is a demonstrable cycle, either in one or the other host. Once demonstrated, the two go hand in hand, as the whole biologic evidence supports the assertion that in the conveyance of protozoa by animal life, but one genus is the rule in such transference. It will be seen, then, that there is a biologic basis for the one medium of contagion, and that efforts for the eradication, transference, prevention, or quarantine would be applied to but two factors—the individual sick with the disease and the genus *Stegomyia.*

The chief aim of the working party was the identification and classification of the specific organism of yellow fever. It was our intention to use every legitimate means to accomplish this end without producing suffering or pain to those under the influence of the disease or in any experimentation that we should deem proper. The bacteriological theme had been well worked over; for our own information it was to be gone over again, especially during the period when the disease could be transmitted to the mosquito.

The blood was to be examined with all modern reagents before the advent of and during the course and convalescence of the disease. All tissues were to be examined histologically after post-mortems to see if they contained either within the cells or in the intercellular substance any organisms or foreign body to which the etiology could be identified. Mosquitoes were to be contaminated by feeding on well marked cases and at varying periods killed and sectioned for histological examination, to determine the presence of a developmental or cycle of maturation of an organism belonging to the *Sporozoa*, and upon the identification of such an organism to give a practical demonstration of its intimate association with the etiology of the disease.

All of this ground had been well worked over by some of the best men of the age with unfruitful results, yet it seemed to us that one technique or another would reveal what we were striving to find; that it was more a question of technique and recognition of something noted before than an actual demonstration in the blood of some organisms that had escaped observation.

An accurate study of the life and habits of the *Stegomyia fasciata* was to be worked out, giving in detail its connection with yellow fever. The other mosquitoes present in Vera Cruz were to be classified and studied in their relation to disease. Such facts of importance that bore directly or indirectly upon quarantine were to be noted and reported.

The result of this work is classified serially.

B.—Sanitary History of Vera Cruz.

The earliest record that we can find showing the presence of yellow fever in Vera Cruz was in 1509, when Diego de Nicues occupied the place, then called New Spain, with 780 men. On the first days of the occupation they lost 400 men; shortly afterwards 200 more; at the end of fifteen months there remained only 60 survivors. From that date the fever has probably continued with variations that correspond to the supply of nonimmunes. The early historical data deals only with great epidemics and correspondingly great losses of life.

During the last thirty-six years, a more or less accurate system of vital statistics has been kept. It is surprising to note that during this time 7,861 deaths have been ascribed to this disease, occurring annually as follows: Estimated population, 38,000.

Year.	Deaths.	Year.	Deaths.	Year.	Deaths.	Year.	Deaths.
1866	264	1876	34	1886	208	1896	0
1867	332	1877	528	1887	4	1897	2
1868	187	1878	448	1888	3	1898	127
1869	10	1879	21	1889	2	1899	670
1870	11	1880	254	1890	40	1900	259
1871	271	1881	723	1891	180	1901	102
1872	215	1882	72	1892	259	1902 (to July 1)	115
1873	322	1883	747	1893	131		
1874	79	1884	136	1894	209		
1875	125	1885	528	1895	143		

During the six months ended June 30, 1902, there were reported 115 deaths from yellow fever, more than one-half of which occurred during our residence there in the last two months. In the month of July there were 30, in August 41, and in September 28 deaths from this disease. Our investigations practically ceased at the end of September.

It would not be amiss to give a general description of the city, with reasons why it has always been a center of infection. Until the last few years the city was built up to within one block of the sea wall.

Three years ago this wall was extended about 200 yards, and on the north carried across the channel, thus connecting the island of Ulua with the mainland, and forming an admirable breakwater for the heavy north seas that break over that coast during the winter months.

The city proper is built as all tropical American cities are. The houses have thick walls, dimly-lighted interiors, and a "patio," or courtyard, in the center. The newer part of the town, where the poor people live, is built mostly of wood and lies to the south of the main town. The sewerage system is primitive, each house or "patio" having a cesspool for the collection of solid excrement. The drainage system for the entire city is superficial; all fluids from the houses and streets run into an open gutter in the center of the street and slowly flow toward the Gulf. The fall of the city is about 4 feet, with the incline toward the Gulf.

The water supply is in keeping with the sewerage system. Many of the older houses still retain their cisterns in some part of the "patio," either exposed or loosely covered with boards. Others have connections with the small water mains, but the great majority, especially those houses occupied by the poorer classes, have no water supply except that caught in barrels and tanks during the heavy rains, or the water from the public street taps carried to the houses in small cans and stored in some convenient uncovered receptacle for the family use.

It will be seen that every house has either one large cistern or a number of barrels and small receptacles, from one to twenty, upon which they depend for the water supply. As we will explain later, the yellow-fever mosquito is purely a domestic mosquito, and finds natural breeding places in such containers. It was an invariable result that an examination revealed from several to many thousand larvæ in every uncovered barrel or tin can. The large cisterns, situated below the ground, gave very few, if any, larvæ. This is accounted for by the numerous other aquatic larvæ and insects that occupy the cisterns, either using the larva of *Stegomyia fasciata* for food, or else a preference of this mosquito for more convenient and better-lighted breeding places.

The social customs of the people are to a large extent conducive to infection and the continuance of yellow fever. The natives of all classes close their places of business and stop work in the middle of the day in order to give their employees about two hours' sleep during the period of greatest heat. This custom is followed by strangers from the day of arrival, and here is the explanation of the immediate infection. The time of flight of the insidious *Stegomyia fasciata* is during the daytime. She finds food in abundance, without resistance, during the hours from 12 to 2, and transmits her infection to all without regard to previous history. Even in Vera Cruz the presence of this mosquito is seldom troublesome, so that the use of mosquito

bars is not demanded or used during the daytime—another factor that aids in spreading the infection.

While the hands and face are usually selected as being the most convenient sites for biting, being exposed, the ankles also come in for a fair share. The use of low shoes and thin socks favor this site, and the practice is condemned as being dangerous during the prevalence not only of yellow fever but also of malaria.

While many foreigners and better-class Mexicans from the "tierras templadas" die annually of yellow fever in Vera Cruz, it may be said that the whole infection is kept virulent and constant by the presence of the native Indians who come down from the mountains to work on the harbor improvements or to sell their native wares. While in Vera Cruz they have no regular domicile, but lodge either in the streets or in large lodging houses, called "patios." These "patios" are seldom without infection; one alone contributed 17 out of 53 deaths from yellow fever in the city during the month of May. It is the Indian mortality that makes the death rate of Vera Cruz excessive. While strong physically, they are slightly resistant to disease, especially yellow fever.

Malaria probably ranks second, tuberculosis third, on the mortality list of the infectious diseases. The infant mortality is necessarily large, death taking place from intestinal disorders.

Outside the infant mortality, due to general bad hygienic conditions, and yellow fever mortality among the strangers, the city is practically free from infections that are so common in our own country; in other words, if the preventable diseases were modified by concerted action on the part of the authorities, we have no hesitancy in stating that Vera Cruz would be as healthy, if not more so, than our average southern city, and as healthy and far more comfortable than any city on the borders of the Gulf and Caribbean sea.

One can not give a general description of the sanitation in Vera Cruz without noting the immense number of black-headed vultures or buzzards that infest every part of the town. They naturally assist in removing from the streets all particles that furnish food for such scavengers.

We are glad to state that the general and city governments are aiding in every way in perfecting a sewerage system, a water system, and in paving and draining the streets of the city. These improvements are inspired somewhat by the results obtained by our own people in the various cities of Cuba and Porto Rico, and when completed they will assist materially in the general sanitation of the city. This work is expected to be completed in about one year.

C.—Synopsis of Previous Investigations.

Nearly every epoch in the medical history of this country has advanced some factor as being concerned in the etiology of this disease. Until

recently various physical phenomena, as well as fruits, air, water, soil, and especially miasms, have been considered as being concerned in its etiology. On the advent of bacteriology much was hoped for, with the following results:

Friere, in 1883, announced the *Cryptococcus xanthogenicus* as the specific agent of the disease, having encountered the organism in the brain, muscles, liver, spleen, kidney, lungs, blood, urine, bile, vomit, and cerebro-spinal fluid. Carmona, of Mexico, in 1885, encountered an organism in the urine of yellow-fever cases at Vera Cruz, with which he associated the etiology of the disease. Gibier, in Habana, in 1887–88, isolated a liquefying organism from the intestinal canal from post-mortems of yellow-fever cadavers, and believed it was connected with the etiology of the disease. Sternberg, in 1889, isolated the bacillus X in about 50 per cent of yellow-fever autopsies. Sanarelli, at Montevideo, in 1897, isolated the bacillus icteroides in about 50 per cent of the cases studied, and for several years it was generally regarded as the specific agent. Wasdin and Geddings, in Habana, in 1900, while investigating the claims of Sanarelli, found the organism in the blood during life and at autopsies in 13 out of 14 cases.

Dr. Carlos Finlay, of Habana, in 1881, advanced the theory of the propagation of the disease by the mosquito, believing at that time that the specific agent of the disease was mechanically transmitted by the proboscis. Later, in 1887, he modified this, having isolated from the body of the mosquito an organism to which he gave the name of *Micrococcus Tetragenus febris flavis*. In 1889 he further modified his theory with what was known as Texas fever, claiming that the mosquito could be infected by feeding upon the discharges of the patient and the disease could be transmitted to the next generation. Finlay, from time to time, has made additional claims in support of his theory, but they were not taken seriously until malaria and its propagation by the various species of *Anopheles* was a well-determined fact.

To the medical board of the U. S. Army, composed of Reed, Carroll, Lazear, and Agramonte, is due the credit of furnishing the scientific proof of the theory advanced by Finlay. In a preliminary report, submitted in 1900, they state that they succeeded in producing a severe and a well-marked case of yellow fever by mosquitoes, which, from two to twelve days previously, had bitten cases of yellow fever on the first and second days of the disease. Before the Pan-American Medical Congress in Habana, in February, 1901, a second report was submitted by Major Reed, in which the preliminary report was confirmed by additional cases and data on the conveyance of the disease by this insect. The conclusions of the board were as follows:

1. The mosquito, *C. fasciatus*, serves as the intermediate host for the parasite of yellow fever.
2. Yellow fever is transmitted to the nonimmune individual by the bite of the mosquito that has previously fed on the blood of those sick with this disease.

3. An interval of about thirteen days or more after contamination appears to be necessary before the mosquito is capable of conveying the infection.
4. The bite of the mosquito at an earlier period after contamination does not appear to confer any immunity against a subsequent attack.
5. Yellow fever can also be experimentally produced by subcutaneous injection of blood taken from the general circulation during the first and second days of this disease.
6. An attack of yellow fever produced by the bite of the mosquito confers immunity against the subsequent injection of the blood of an individual suffering from the nonexperimental form of this disease.
7. The period of incubation in 13 cases of experimental yellow fever has varied from forty-one hours to five days and seventeen hours.
8. Yellow fever is not conveyed by fomites, and hence disinfection of articles of clothing, bedding, or merchandise, supposedly contaminated by contact with those sick of this disease, is unnecessary.
9. A house may be said to be infected with yellow fever only when there are present within its walls contaminated mosquitoes capable of conveying the parasite of this disease.
10. The spread of yellow fever can be most effectually controlled by measures directed to the destruction of mosquitoes and the protection of the sick against the bites of these insects.
11. While the mode of propagation of yellow fever has now been definitely determined, the specific cause of this disease remains to be discovered.

In July, 1901, an additional report was submitted by Reed and Carroll, in which it was shown that yellow fever could be transmitted by the direct inoculation of blood from a case of yellow fever to a nonimmune individual. They also made a series of investigations which tended to prove that the whole blood was not necessary to produce the disease, but by first using serum and later using serum diluted with normal salt solution and filtered through a Berkefeld filter the infectious principle would also be transferred and cause the disease as readily as with the whole blood.

3. EXAMINATION OF THE BLOOD AT VERA CRUZ.

A.—Examination of Fresh Blood.

A review of the literature of recent years shows that changes in red blood cells in yellow fever can not be expected. The older writers mention various disintegrations in the corpuscles that probably resulted from the poor technique of their period.

The procuration of blood specimens for examination should be done with the most rigid cleanliness, both as to the slides and cover glasses and patient, at the point selected to procure the blood. It has been our custom to use new slides and cover glasses, recently cleaned in a mixture of nitric acid and alcohol and kept in alcohol until used.

To procure dry films for staining, slides warmed to a temperature of about 40 degrees C. were brought in contact with a small drop of blood as it flowed from the wound and evenly spread in a single layer by a glass-rod spreader.

Red blood cells.—Red blood cells from a case of yellow fever, freshly spread and immediately examined, presented no visible variation from the cells of a normal individual. The size and form of the cell are normal; a few more shadow corpuscles are seen than are observed in normal individuals in temperate climates, but this was noted generally in nearly all healthy subjects living in the Tropics. No pigment could be observed within these corpuscles. The staining reactions were those ordinarily observed in health. A number of counts were made in order to observe whether there was any destruction of red blood cells at the beginning of the disease and as it progressed. In cases where the count was made on the first or second day, and then in the later days, a progressive increase in the number of red blood cells was noted from an average 4,600,000 to as many as 6,000,000. It is believed, however, that this increase is relative, being due to dissipation of fluids. In experimental case No. 1, where the count was made before and during the progress of the disease, their increase is noted.

Hæmoglobin.—A number of estimations were made with the Dare's hæmoglobinometer, at the bedside of the patient, and with this instrument a percentage of hæmoglobin proportionate to the number of red blood cells was observed in nearly every instance. In the later days of the disease the increase in the hæmoglobin was proportionate to the increase of the red blood cells. The average percentage of hæmoglobin in healthy individuals in the Tropics is about 80 per cent.

White blood cells.—As far as we could ascertain, there was no variation from the normal white blood cell in structure, pigment, vacuoles, or fat. There was, however, a marked variation in the granulation caused by the complete or almost complete disappearance of the eosinophile cell. This is due to the rapid and almost complete destruction of the hepatic cell, whereby the glycogenic function is seriously interfered with. It has been our experience that the percentage of eosinophiles, when present, was more or less proportionate to the destruction of the liver cells, with consequent variation from normal in rare instances to complete disappearance in the majority of cases. No pigmentation is seen in white blood cells in typical cases of yellow fever.

A differential count was made in a number of instances from typical cases, without presenting more than a normal variation. The white blood cell from a case of yellow fever reacts the same to stains as a normal white blood cell. The cells containing neutrophilic granules seem more conspicuous, probably on account of lack of variation with basophiles and eosinophiles, the latter being absent and the former not reacting to the polychrome stain used.

Blood platelets.—It is to be regretted that there has been little advance in our knowledge of these blood elements since the original descriptions of Bizzozero and Hayem. In the last few years staining methods have been devised which reveal structure within these bodies

hitherto undescribed. Our examination of the blood of yellow-fever cases has shown these bodies possessing properties which we have only recently demonstrated in normal blood.

While examining our fresh blood spreads from these cases, the described blood platelet was seen with its irregular borders lying free and singly in the plasma or in aggregations of from four to ten or more in irregular clumps and apparently attached to one of the glasses. In a short time, from fifteen to thirty minutes in that climate, the platelet began to lose its irregular outline, became larger, round, and more distinct by increasing its refractility, and then to establish within itself a granulation of its protoplasm, which assumes a rapid dancing motion to and fro across the corpuscle. These motile granules within the cell are due to a substance having a high index of refraction, thus shutting off the speck of light when in the proper focus. The number of these granules vary from four to twenty, depending on the size and changes in the platelet, as yet unknown.

The platelets arranged singly go through the cycle more rapidly than those grouped in clusters, though these eventually present the same changes.

Some slides were watched under the microscope to see how long these granulations continued in motion. There is but little change at the end of twenty-four hours, but without using warm stages we found the motion to cease in forty-eight hours, though there was no disintegration of the cell.

The size of these platelets vary considerably from 1 to 8 micra. All sizes can be easily distinguished by features more or less common throughout the various sizes. The granules in those of small size are consequently very difficult to see.

The presence of a body in yellow fever presenting more or less the general features of some of the *sporozoa* was suggestive enough to make comparative studies in normal subjects, as well as those suffering from the kindred disease, malaria.

In normal subjects, immune and nonimmune, living within the Tropics it was seen that this same change was effected, though more slowly, under the same conditions as in the examination of yellow-fever blood. In the examination of malaria it was discovered to be almost impossible to find them. This, however, corresponds to the known clinical data of that disease, the platelets being considerably diminished in numbers. Some of the platelets when seen undergo the same modification with the formation of granules as in yellow fever and in normal residents in the Tropics.

Staining methods were applied to bring out, if possible, the finer structure of these bodies. All combinations were used, but only those modified after Romanowsky give the desired results. In a specimen of yellow-fever blood in our possession that was promptly fixed and

stained with a modification of Romanowsky's method these bodies were seen with their irregular outlines. The protoplasm stained a faint blue; their contents shows what appears to be the granules stained, a deep eosin pink in sharp contrast to the protoplasm. The number of these granules could be estimated with more or less accuracy in this preparation; the number, we may say, is from four to twenty. In a severe malignant quotidian malarial infection, this same staining peculiarity was noted, though, as stated above, the platelets were greatly diminished in numbers.

While we had made many blood examinations, it was not our experience to observe platelets undergoing these changes in normal blood, or in the blood of other diseases, prior to this time. As soon as we returned to the States a series of studies was undertaken, and it was soon seen that under similar conditions of light and heat the same granular condition of the blood platelet is affected in normal blood at the expiration of one hour as we had observed taking place almost immediately in the blood of yellow-fever cases. Staining reactions were applied, using the same technique as that employed in Vera Cruz, with the following results:

First. In which the platelet stained a pale blue at the edges, with a well-defined though diffuse purple area in the center.

Second. In which the platelet partook of the type found in yellow-fever cases and in about the same proportion.

It is not known what influence makes this stain variable, whether it is due to the stain, its method of application, or whether there are two distinct types of platelets.

Plasma.—The plasma was examined, both in the fresh state and in the dried film, after appropriate straining. Every technique known to us was used, but the results were negative. In these examinations many structures of various shape were observed from time to time that we were not familiar with, but they were not encountered with sufficient regularity to study in detail.

B. Bacteriologic Examination of the Blood during Life.

Technique.—Specimens of blood were taken from two localities, the ear, and the median basilic vein, at the bend of the elbow, in each instance after surgical cleanliness. The skin of the ear was pricked with a sterile needle, the blood collected in sterile pipettes and immediately planted. From the median basilic vein the blood was withdrawn by means of a sterile hypodermic syringe and immediately planted. Ordinary bouillon was used as media in quantities from 10 to 100 c. c., using as a rule 2, 5, 10, and 30 drops of blood in the various flasks. Virulent cases were selected from time to time and a series of tubes and flasks inoculated. In selecting these cases particular attention was paid in getting otherwise well-formed and healthy young

adults from the first to the last day of the disease. In all of these so selected the results were uniformly "no growth."

In the first case, that of an old man then in the agonic period, an organism belonging to the colon group was isolated. This organism gave gas production with glucose, negative with lactose; produced acid, but did not coagulate milk (the milk was of very poor quality), and gave a pronounced indol reaction. This was the only organism isolated from the blood during life, and as the subject was old and practically disorganized from age and disease, the organism, outside of its presence, had no significant bearing on the disease.

It has been the general experience of investigators that vegetable organisms are not demonstrative in blood smears of yellow fever cases. We concur in that opinion.

C.—Serum Agglutination with the B. icteroides, B. typhosus, B. dysenteriæ, and B. coli communis.

In order to test the agglutinative power of yellow fever serum, four organisms were selected from the stock of the Hygienic Laboratory. These organisms were the *B. icteroides*, of Sanarelli; *B. typhosus*, *B. dysenteriæ*, Shiga, and the *B. coli communis*. All of these organisms had reacted typically to their species.

Two methods of applying the reaction were used in each case: (1) After the method of Widal; (2) after the sedimentation test in small test tubes. All four organisms were applied to each of ten typical cases, in dilutions of 1–40, and in every instance the result was negative, both under the microscope and in the test tube.

These observations are in accord with other recent data on the specific power of yellow fever serum in its relation to the group of cocco bacilli.

4. BACTERIOLOGIC EXAMINATION OF ORGANS AT NECROPSY.

This subject was not gone into in detail more than to convince ourselves that with ordinary working media, inoculations from the spleen, liver, kidneys, and heart's blood of virulent uncomplicated cases, no organism is encountered with sufficient regularity to suggest a fulfillment of Koch's postulates. At every autopsy where cultures were taken we satisfied ourselves that death had taken place within one hour. Inoculations were made into bouillon and on agar slants, and every uncomplicated case gave uniformly "no growth." In one case with marked hemorrhagic infiltration of the lungs a diplococcus that retained the stain after Gram's method was encountered in the blood and organs, with the exception of the kidneys.

In a mosquito killed three days after having fed on this patient an examination of the contents of the stomach showed it to be filled with

partially digested blood in which a large number of these same diplococci were present. In the case from which we isolated a colon organism before death the same organism was isolated after death from the blood and organs.

To summarize, our experience was that in typical uncomplicated cases in young adults, the autopsy being performed immediately after death, the blood and organs were found to be free from vegetable organisms.

We therefore ceased our examinations of the blood and organs and turned our attention to the *Stegomyia fasciata* and a more complex series of organisms, the *protozoa*.

5. THE STEGOMYIA FASCIATA (FABRICIUS).

Stegomyia fasciata (*figs. 1 and 2*).—This mosquito was first described in 1805 by Fabricius under the name *Culex fasciatus*. Since then it has been given no less than seventeen different specific names under the same genus, the more common being *Culex tæniatus* and *Culex mosquito*. Theobald in his recent monograph has transferred this insect to his new genus *Stegomyia*, under the name *Stegomyia fasciata*. As there is no better description than that given by Theobald, he will be quoted verbatim:

Female.—Head densely clothed with broad, flat scales, black and gray on each side, a white patch in the middle, in front, extending back to the neck, a white patch on each side, a thin white border to the eyes; the scales at the back of the crown with an ochraceous tinge in some lights, long black bristles projecting forward; eyes black, with silvery patches in some specimens; antennæ blackish, with narrow, pale bands; basal joint black, with a patch of white scales on the inside (appearing as two small white spots with a lens); second joint sometimes pale testaceous at the base, pubescence and verticils dark brown; palpi black-scaled, the last joint with pure silvery white scales inside and on the tip, sometimes entirely white.

Thorax dark brown, covered with reddish brown, pale golden and creamy curved scales, ornamented as follows: A pure white, broad curved band on each side, curved inward about the middle of the mesonotum and continued back as a thinner pale line to the scutellum, two thin parallel pale scaled lines between, extending about halfway across the mesonotum and more or less on to the scutellum, a short white line in front between these two, a white spot on each side of the thorax in front near the neck; scutellum with a thick row of white scales and with three tufts of bristles; metanotum brown; pluræ dark brown with several patches of silvery scales.

Abdomen dark-brownish black, with basal bands of white scales; first segment densely clothed with creamy scales and edged with pale hairs; sides with patches of white scales forming more or less flat triangular patches.

Legs with the femora with the bases yellowish, dark scaled toward the apex, extreme tip pure white, ventral surface partly covered with white scales; tibiæ black; metatarsi with basal white bands; fore tarsi with the first joint basally white, rest black; mid tarsi the same; hind tarsi all basally white, except the last joint, which is pure white, penultimate joint mostly white with black apex; fore and mid ungues both toothed and hind without teeth.

Wings with the veins clothed with very long, narrow, brown scales and short,

median, broad, dark-brown ones; first submarginal cell longer and but slightly narrower than the second posterior cell, base of the former a little nearer the base of the wing than the latter; posterior cross vein about one and a half times to twice its length distant from the mid cross vein.

Halteres ochraceous, sometimes the knob is slightly fuscous.

Length: 3.5 to 5 mm.

Male.—Darker than the female.

Head black with white scales in front and in the middle; antennæ brown with paler brown bands, sometimes almost white, basal joint jet black with a large tuft of pure white scales, plumes brown; palpi black with four white basal bands; proboscis black; thorax marked as in the female but much darker, and the white scales clearer and more silvery.

Abdomen with the first segment with creamy scales, bases of the second to the fifth segments white, fifth to eighth with white lateral spots; these spots also occur on the front segments, but quite at the sides.

Legs as in the female. Fore claws unequal, the larger one with a short, blunt tooth, smaller one untoothed; mid ungues unequal, untoothed; hind equal, untoothed.

Length: 3 to 4.5 mm. (Theobald.)

Habitat.—This mosquito is widely distributed, probably more so than any other species, being found throughout the tropical world and well up into the temperate zone. At one time it was supposed to be a coast mosquito, but now it is found to have spread along the commercial lines of communication to cities in the interior that furnish receptacles for breeding places. Its acclimation to the altitudes is gradual, as may be illustrated by the following example:

About twenty-eight years ago a railroad was constructed connecting Vera Cruz with the City of Mexico. Some years later a competing line was built between these cities, but going through a different part of the country. Along the line of the Mexican Railroad yellow fever was unknown in the interior. During the construction of the railroad the disease prevailed among the employees until the road reached the foothills; it then disappeared. About nine years ago yellow fever appeared in Cordoba at about an altitude of 3,000 feet, and has since been epidemic. Three years ago yellow fever appeared at Orizaba and this year (1902) there was a severe epidemic in that city. Since the construction of the railroad many cases of the disease had been received during sickness and convalescence, and many cases have developed among strangers going through Vera Cruz to that city without the disease in any way affecting the general health of the community, until three years ago.

In the second instance, along the line of the Interoceanic Railroad we know by actual observation that the *Stegomyia fasciata* has been ascending from station to station until it has now reached Carasal at an altitude of about 3,000 feet. Synchronously with the ascent of this mosquito yellow fever became epidemic in those places. El Palmar, the next station above Carasal, about 8 miles distant, does not harbor any of these insects at the present time, and though some cases were sent there from the latter station this last year, there was no

spread of the infection. Jalapa, a city of about 35,000 inhabitants, at an altitude of about 4,500 feet and about 20 miles above Carasal, does not harbor the *Stegomyia fasciata*, and though cases have been sent to that place for years from Vera Cruz and intermediate stations, there has never been any spread of the disease.

It will be seen in these instances that the ascent of yellow fever and the advent of the *Stegomyia fasciata* have gone hand in hand until they have now reached an altitude of 4,200 feet in the first instance and 3,000 feet in the second. This is the first authentic record we have of yellow fever reaching such an altitude and is another proof that, the proper conditions supplied, the disease and the insect can be introduced and cause the same destruction of life as in more tropical regions. The distribution of this insect in our own Southern States has already been dwelt upon.

In regard to the spread by ships, our observations were limited. They were only found breeding in one schooner, which subsequently went to Ship Island quarantine, badly infested with the insect. Two more instances are reported by Passed Assistant Surgeon Grubbs at the same station where the adult insect traveled on wooden vessels in numbers from the same place. In an examination of a number of iron ships this insect in its larval stage was not encountered, but it was found in the adult stage in numbers depending upon the proximity of the ship to the breeding places. Giles reports an instance where he has known this mosquito to make a voyage from Kurachi to Suez, speaking as though the transference had been made in an iron vessel. We believe such transfer by iron vessels is rare and could only be accounted for by the insects prevailing in immense numbers and the ships lying almost in touch with the residence part of the port. In the transfer by sailing vessels the conditions are different, as on these ships there is always water conveniently stored on some part of the deck suitable for breeding places, and two or three females will in a short time, through their progeny, infest the vessel. The development of the larvæ and pupæ seems to be certainly prevented aboard ships at sea by the constant agitation of the water in the containers.

Time of flight.—We may say that the flight of this mosquito is restricted to the hours of sunshine. It was taken by us occasionally, in a well lighted room as late as 9 p. m.; generally speaking, however, the period of greatest flight is in the early morning and late in the afternoon. While we speak of the flight as during the daylight, it is, of course, relative and applies only as a generality. There are instances where the insects may be encountered at other times, but not in sufficient numbers to say that it is a habit of the species. If the presence of several hundred of this species in a jar in captivity is an indication of their natural habits, it can be said that the time of flight is limited to the daylight, for when night approaches the activity of

the insect ceases, while during the daytime they are exceedingly active.

The approach to attack of *Stegomyia* is extremely insidious, usually approaching an individual on the shady side and without warning. The pertinacity of this mosquito in returning to its victim after it has been repeatedly driven off is almost characteristic. It has been noted repeatedly by us that females escaping while being transferred from one jar to another would almost unfailingly return after a few minutes and attempt to secure food from one of us. On one occasion a marked female was driven off five times during one forenoon; once she remained away nearly an hour, when her intended victim becoming tired of further timekeeping, finally recaptured her. Having satisfied its desire for food it gives vent to a triumphant note and goes in search of a resting place. It is generally agreed that an albuminous diet is essential for the maturation of the ova. The time between the acquisition of food and the complete maturation of the ova may be said to be from three to six days. At the end of this time the instincts of the insect are for ovipositing, but being prevented she seeks more food and lives on indefinitely until this act occurs As will be referred to later on, it has been noted by us that the ova of mosquitoes contaminated by biting a case of yellow fever undergo degeneration, which probably accounts for the extended life of contaminated mosquitoes of this species.

Aside from the daily variation in the flight of this insect, there are seasonal prevalences which must necessarily vary with the temperature and rainfall of any particular locality. The flight of *Stegomyia* is in Vera Cruz an almost uninterrupted one throughout the year. On our arrival in the city in the early part of May it was very numerous; in fact, there was no appreciable increase throughout the subsequent two or three months. In July, however, frequent rains kept flushing the barrels, washing out a great many larvæ and pupæ, which apparently perished. The decrease of *Stegomyia* at this time was noticeable; with it also came quite a reduction in the number of yellow fever cases. From the statistics of yellow fever in Vera Cruz it is evident that the disease almost disappears in some years during December, January, and February, which, no doubt, must be attributed to the fact that even in that climate the species is forced to retire from full activity for some time. With the reduction of the disease transmitters there is implied and was actually noted in July and August a decrease in the number of cases of yellow fever.

We must recall the fact, however, that most diseases at the beginning and subsidence of epidemics are usually milder than during the height of the epidemic, so that we are inclined to believe that in the colder months, while there is an actual reduction in the number of cases, there is also a great decrease in the severity of the symptoms, so that

probably many cases are not recognized. Again, like all other insects, *Stegomyia fasciata* is often more common one year than another, a circumstance which is possibly explained by the fact that they, like certain low organisms, require a period of rest; in other words, after enjoying one or two or even three or four years of full flight, the reproduction of the species becomes diminished, which for recuperation may require an equal period.

The character of the breeding places has been sufficiently dwelt upon. Any uncovered receptacle suffices for this purpose. The eggs (fig. 3) are deposited regularly, after the manner of the genus *Anopheles*, and may vary from 40 to 150 in number in a single brood. The eggs are small, about 0.75 mm. in length to 0.04 mm. in breadth. They are black in color, and are enveloped by a thin transparent membrane, which under a magnifying glass gives them a silvery appearance. In shape they are elongate, a little flatter on the upper than on the lower surface, upon which they float on the water like a miniature boat. The young larvæ hatch by dehiscence of the broader end in from ten to twenty-four hours.

Larval stage (fig. 4).—The young larvæ escaping from the eggs commence now an existence which in activity and voraciousness is scarcely attained in any other mosquito known to us at present. They never seem to rest, nor are they content to await their food like other species. They scour the water in every direction, and many times we have wondered why these larvæ seem to be larger and stronger the cleaner and freer from microscopic growth the can or barrel would be. The larvæ of *Stegomyia fasciata* are readily identified from those of other species by the shape of the respiratory siphon, which is black and barrel shaped and much shorter than in the *Culex pungens* or others. The color of the body also is different; for while the general color effect of *Culex* larvæ is usually grayish-brown, the larvæ of *Stegomyia* are whitish-gray and when nearing maturity almost milk white. In order to determine the identity of the larvæ it is necessary to dip them out of the water with a glass, when the difference in the siphon and the color may be seen at a glance.

The larvæ are full grown in from eight to ten days and then change into dark-brown pupæ (fig. 5). The identification of the pupæ is somewhat more difficult than that of the larvæ. Nevertheless, after a little experience a glance suffices to determine the pupæ of *Stegomyia fasciata* from those of other mosquitoes, and especially from those which may be found in the same water with them. The chief characteristic is the narrower and more graceful thoracic portion, which in the *Culex* species is comparatively clumsy in appearance. The respiratory siphon of the pupæ being slightly posterior to that of *Culex*, the body assumes a position horizontal with the surface of the water, with less curvature of the tail than has the genus *Culex*. The color of the

pupæ is much darker brown in *Stegomyia*; but, after all, the identification of that mosquito is just as well made from the larvæ, for they will be found at the same time with the pupæ. The complete imago escapes from the pupa case in from one to two days. The entire metamorphosis is shorter in *Stegomyia* by two to three days than in *Culex* and is completed in from eight to ten days. The difference of time in each species, of course, depends upon the temperature and supply of food.

In their characteristic breeding places the larvæ and pupæ have one trait that is of interest in connection with their destruction. When the barrel or other container with thousands of larvæ is approached and slightly agitated, the insects disappear rapidly to the very bottom, so that nearly all the water can be dipped from the barrel without removing more than a few of the larvæ. The barrel may be turned upon its side, and it will be found that about 80 per cent of the larvæ will stay in the few remaining ounces of water. In view of the fact that the greater percentage of larvæ and pupæ remained after the emptying of the barrel, it was recommended that nearly all the water be poured off and the remainder kerosened, or that the barrel be washed repeatedly to free it from larvæ. It is worthy of note that pouring water containing the larvæ of *Stegomyia fasciata* on the ground is usually as rapidly fatal to them as though they had been kerosened.

A BRIEF DESCRIPTION OF THE ANATOMY AND HISTOLOGY OF THE ADULT STEGOMYIA FASCIATA.

No attempt will be made to give a detailed description of the anatomy of the insect. Only those structures or organs that bear directly upon the investigation will be described.

The histology of the salivary glands.—Each salivary gland consists of six tubules, three in each lateral half (fig. 6). The tubules of each half of the gland unite to form the right and left salivary ducts, which soon unite with each other to form the common median salivary duct, which empties into the buccal bulb. Each tubule of the salivary gland consists of a single layer of polyhedral or short columnar cells with a large, long, central acinus. The nuclei lie usually toward the basement membrane. The central tubule of each lateral half is straight, while the remaining two, one above, the other below, are more or less convoluted, especially toward the free extremity (fig. 7). The cells of the *Stegomyia fasciata* glands in uncontaminated mosquitoes are uniformly clear, almost vesicular, thus differing from the granular type frequently met with in uncontaminated species of *Anopheles.*

The ducts of the salivary glands are reenforced with an annular arrangement of the chitin somewhat like the spiral arrangement in the tracheæ. The lining membrane consists of a single layer of epithelial

cells attached to a basement membrane; the nuclei of the cells lie close to the basement membrane.

The alimentary canal is divided into a fore-gut, mid-gut, and hind-gut, with the following subdivisions:

Fore-gut	Mouth. Pharynx with pumping organ. Esophagus. Esophageal diverticulum.
Mid-gut	The homologue of the proventriculus. The stomach. The pylorus.
Hind-gut	The pyloric dilatation. The small intestine. The colon. The rectum.

Fore-gut.—The mouth and pharynx are lined with chitin; the esophagus and esophageal diverticulum consist of a thin membrane composed of flat cells, extending from the pumping organ to the proventriculus above and down into the abdominal cavity ventrally (the diverticulum) (fig. 8).

Mid-gut.—The mid-gut extends from the proventriculus to the origin of the Malpighian tubules (fig. 9). The anterior portion of the midgut is narrow, the posterior dilated. Both are lined with a single layer of short columnar cells with nuclei almost in contact with the basement membrane (fig. 10).

The pylorus approaches a true valve structure by an apparent invagination of the epithelium into the stomach.

Hind-gut.—This part of the intestine is lined throughout with short columnar cells with large nuclei. In the pyloric dilatation the five Malpighian tubules enter. The small intestine presents a series of convolutions, dilates to form the colon, and later to form the rectum. In the rectum are seen six papillæ, the rectal glands (figs. 11–12).

The reproductive system.—This system consists of the—

1. Ovaries.
2. Oviducts.
3. Mucous glands.
4. Spermatheca and ducts.

In the young adult female mosquito the ovaries are attached to the fourth and fifth abdominal segments. As they become mature after fertilization and an albuminous diet they gradually occupy the whole of the posterior abdominal segments (fig. 13).

The balance of the reproductive system is not of interest in connection with this investigation.

The muscle plates of the thorax consist of faintly striated muscle cells with nuclei immediately beneath the sarcolemma arranged in rows (fig. 14).

6. THE CONTAMINATED STEGOMYIA FASCIATA AND ITS PARASITE.

In order to secure contaminated mosquitoes for examination, about twenty large young females should be selected from the breeding jars and placed in a specially prepared cage, made with three sides of wire gauze and one side of glass for observation. A cheese-cloth sleeve at the top makes the mosquitoes accessible and permits the patient to place his hand inside the cage.

After the mosquitoes are placed in the cage it is set aside for several hours, so that they will become hungry. The cage is then taken to the bedside of the patient, his hand introduced and permitted to remain therein until the mosquitoes have gorged themselves with blood. The time required for this is variable. As a rule it may be stated that all that are going to feed will do so within thirty minutes. The cage is then taken to the laboratory and placed in a subdued light for twenty-four to thirty-six hours without the addition of food of any kind. At the end of this time it will be found that all of the mosquitoes that did not feed have died.

A few male insects should be added to the cage and artificial feeding begun. Three articles may be used as food: (1) The blood of those sick with the disease, (2) bananas or other overripe fruit, and (3) cotton well moistened with a thin sirup. The first and second answer best, with preference for the first.

With this technique mosquitoes can be kept alive for several weeks.

Previous to killing they should not be fed for one or two days, as the stomach will be found so thin by distension that satisfactory examination is impossible.

From almost every case studied in detail a large number of insects were contaminated and their killing commenced on the second day and continued at stated intervals until between the twentieth and thirtieth day. After killing they were embedded in paraffin, cut serially, and appropriately stained. While contaminations were made from cases of yellow fever as late as the seventh day of the disease, most of our attention was given and practically all our results were obtained from mosquitoes contaminated during the second, third, and fourth days. The results of the examination of mosquitoes contaminated on different cases were fairly uniform, a large proportion of the insects presenting one or more stages of development of a well-defined animal parasite.

Sections of mosquitoes killed two or three days after contamination showed the stomach distended with undigested blood. In this no organism could be demonstrated to our satisfaction. With the disappearance of the blood on the third day after feeding by the process of digestion a parasite became plainly visible and could be traced though the following stages with more or less accuracy.

Beginning, then, with three or four day mosquitoes, after the disap-

pearance of the blood, we observe in the stomach cavity varying numbers of small fusiform-shaped protozoa (fig. 15), lying principally in groups, though many are single. These fusiform-shaped bodies are well defined, with an oval central nucleus. In studying the groups we noted certain bodies which bear a striking resemblence to cases of terminal conjugation such as are described for certain sporozoa. While these conjugating bodies are noted principally in the stomach cavity, they are not necessarily confined to that organ; we find in two specimens having a large number of such bodies that they have passed through the stomach wall and are undergoing the same apparent conjugation in the esophogeal diverticulum where subsequent changes take place.

The stomach wall has undergone some modification during this process. It has become hypertrophied and shows many bodies similar in shape to those found in the localities mentioned, but naturally can not be so readily identified as when lying free. However, as they are found only in the stomach and diverticulum we know of nothing that could cause us to err if we say they are the same. We may say, then, that after this apparent conjugation the resulting body (zygote) passes through the stomach wall into the esophageal diverticulum. We then find in the diverticulum (preserved specimens in microtome sections) a mass of material the nature of which is at present not entirely clear to us. It has more or less the appearance of an albuminoid mass, but its origin and exact nature still remains to be explained. In this mass, more particularly at the periphery, we observe further developmental stages of the parasite; the latter has increased in size, its nucleus has undergone fragmentation or multiple division (fig. 16). The resulting chromatophilic granules from this division now rapidly increase in size, become rather sharply defined and more or less regularly elongate oval, the "sporoblasts?" (figs. 17 to 24).

The "sporoblasts?" are more or less definitely limited in the early stages by a membrane. Later this becomes so thin as to be imperceptible, though the aggregations of "sporoblasts?" still retain their well-defined areas until the disintegration of the encysting albuminoid mass in which the development takes place. The number of "sporoblasts?" in each "oöcyst?" is rather constant, thirty or forty being visible in each median section. As the albuminoid mass disintegrates and the "sporoblasts?" become mature the latter are liberated (figs. 25-26).

In order to appreciate the phases that are to follow, the anatomy of the esophageal diverticulum must be clearly comprehended. A reference to figs. 8, 9, and 11 will show the relations of the diverticulum. Beginning at the homologue of the proventriculus, the diverticulum passes downward in contact with the anterior constricted part of the midgut. Here it is surrounded by the six tubules of the salivary gland, three on each side, and in contact with it through the intervention of a delicate reticulum of connective tissue.

The position of the posterior or dilated portion of the midgut (the so-called stomach) depends upon the state of distention of the diverticulum, it being in contact with that structure superiorly (dorsad) as far down as the pyloric dilatation and inferiorly (ventrad) with the connective tissue of the abdominal segments, so that a protozoan parasite passing through the midgut in the direction of least resistance (ventrally) enters this diverticulum and undergoes future stages of development. In malaria the sporozoite formation is said to take place in the stomach wall of the mosquito, but this we believe to be an error in interpretation, the significance of this diverticulum, its unobstructed connection with the thorax, and intimate relations with the salivary glands not being thoroughly appreciated.

After the liberation of the "sporoblasts?" migration commences in the direction of least resistance, which is, as stated above, toward the thorax. Arriving at the anterior (cephalad) part of the diverticulum they are, with the exception of that thin structure, in contact with the salivary glands on all sides except above (dorsad), the upper border being occupied by the anterior constricted part of the midgut. The "sporoblasts?" then penetrate the diverticulum and become disseminated throughout the connective tissue supporting the gland (figs. 27, 28). The "sporoblasts?" then enter the gland structure and gain access to the salivary cells, where another important phase takes place. This phase consists in the breaking up of the resting "sporoblasts?" (which now apparently become the spores) into a number of infinitesimally small "sporozoites?" (figs., 29, 30), which ultimately are freed, find their way into the lumen of the gland, and would naturally be discharged by the insect in the act of procuring food.

We have now followed and illustrated four important and pronounced stages of an animal parasite which we have encountered in mosquitoes fed on cases 11, 21, 36, and in some scattering cases studied before the organism appealed to us as probably being connected with the etiology of yellow fever. We have produced yellow fever experimentally with mosquitoes from case 36 that harbored this parasite and have again found the parasite in mosquitoes fed on this case.

As further evidence we permitted this same species of mosquito, *Stegomyia fasciata*, to feed on a case of malarial fever of the malignant quotidian type, on normal blood, and on sugar and water. *In none of these mosquitoes did we find the parasite described above.*

Systemic position.—From the description given above it is clear that we are dealing with a sporozoön which in all probability is passing through its sporogenetic (sexual) cycle. The stages found remind one very strongly of the corresponding cycle of the malarial parasite, and the inference would not be unnatural that the schizogonic (nonsexual) cycle would be similar to that of *Plasmodium*. Still, not only we, but Sternberg, Reed, Carroll, and others have made careful

studies of the blood in cases of yellow fever without finding any structure which could be legitimately interpreted as the schizogonic cycle of the parasite now under discussion. Until that cycle is found a definite systemic position of the order or family of the Vera Cruz organism is scarcely possible. We do not see how it can be legitimately united generically with any of the sporozoa thus far described, and on this account, and especially because of its probable importance in connection with yellow fever, we think the action of proposing for it a new generic name is not unwarranted. Pending further investigation we suggest that the parasite be provisionally placed in the Hæmosporidia, but we frankly state that this proposition is one based entirely upon practical considerations of convenience rather than upon a conviction of its exact position.

As generic name we propose—

MYXOCOCCIDIUM Parker, Beyer, Pothier. 1903.

Generic diagnosis.—Ordinal position uncertain.?? Hæmosporidia: *Schizogonic* stage unknown. *Sporogenic* stage: A fusiform stage 3 to 4 μ long by $1\frac{1}{2}$ to 2 μ broad, in the lumen of the stomach and esophageal diverticulum, seen about three days after mosquito has bitten yellow-fever patient; nucleus present. A globular stage (?oöcyst) in the esophageal diverticulum, imbedded in an albuminoid mass of undetermined origin and nature. This reaches maturity and breaks up into numerous elongate-oval bodies (?sporoblasts) 3 μ long and 2 μ broad, which enter the cells of the salivary gland, where, coming to rest (?spores), they break up into numerous excessively minute (?sporozoites).

As specific name we propose STEGOMYIAE, on account of the organism being first encountered in this genus of mosquito—

MYXOCOCCIDIUM STEGOMYIAE Parker, Beyer, Pothier. 1903. (Figs. 15 to 31.)

Specific diagnosis.—*Myxococcidium.*—Same as generic diagnosis.

Habitat.—The yellow-fever mosquito, *Stegomyia fasciata*, in Vera Cruz, Mexico.

Type specimens.—In collection of the Hygienic Laboratory, U. S. Public Health and Marine-Hospital Service.

The examination of other structures in contaminated mosquitoes.—The other organs of the mosquito present evidences of marked alteration, both in structure and function. Beginning with the ovaries of a mosquito fed on normal blood, we find the ova undergoing immediate hypertrophy, distending the whole abdominal cavity (fig. 13), pressing the other organs forward and downward. In mosquitoes contaminated by feeding on a case of yellow fever the ova primarily take on a hypertrophy, but subsequently begin to degenerate until there is practically nothing left but fibrous tissue (figs. 31, 32). We have attached considerable importance to this, believing that this degeneration alters the natural habit or instinct of the insect and prolongs its life for some time, owing to the suspension of the process of ovapositing. No parasite of any kind, however, could be demonstrated undergoing development in these ova.

The salivary glands of a normal mosquito are presented in figs. 6 and 7. They undergo considerable change in contaminated mosquitoes. There is at first a slight hypertrophy of the glands of mosquitoes fed on normal blood, but in those fed on yellow fever the glands undergo excessive hypertrophy and become convoluted, forming large masses of gland structure (figs. 33, 34) rather than the individual tubules seen in normal blood-fed insects. Outside the simple addition of the spores and sporozoites no structural changes could be noted in these organs.

7. EXPERIMENTS.

In order to have a demonstration of the transmission of yellow fever by the mosquito, to study the changes that are present in the tissues of the mosquito and to study the changes in the blood of the subject, it was decided to attempt the inoculation of an individual.

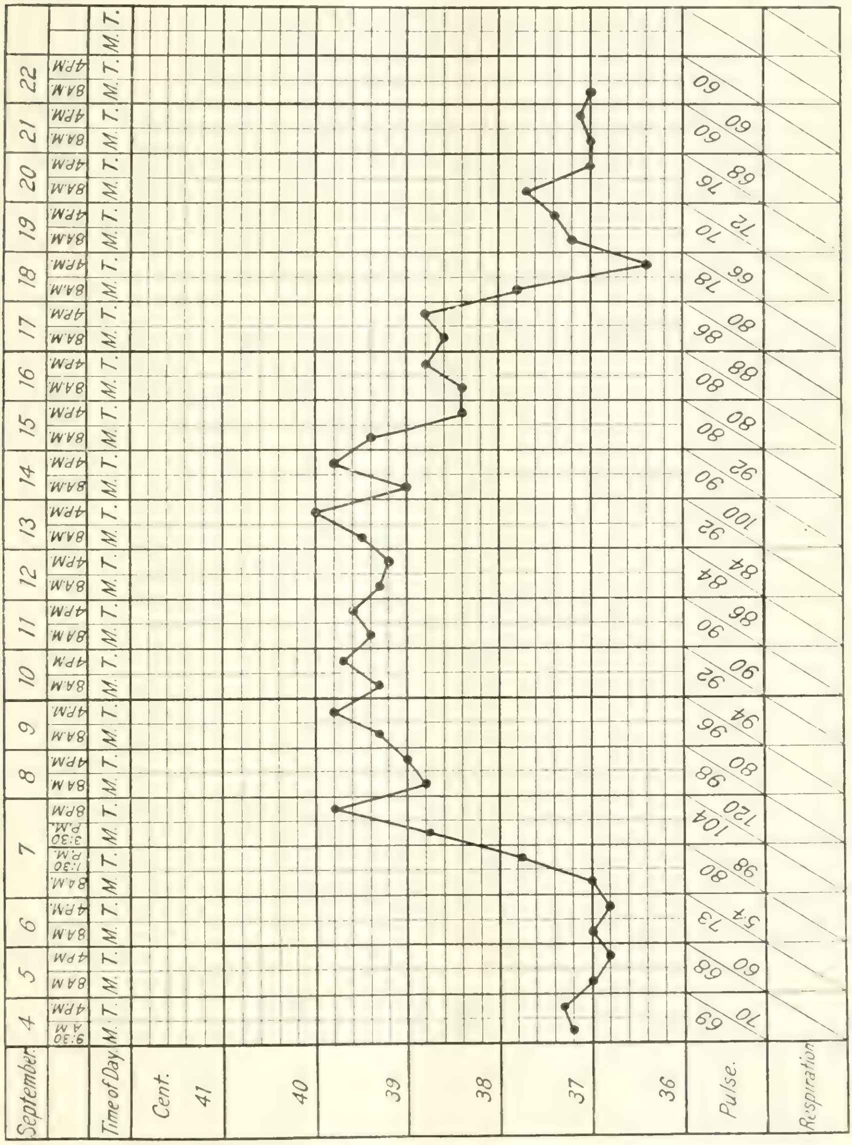
A room was made sufficiently mosquito proof and a man brought from Jalapa, a town of 4,500 feet altitude and as yet free from yellow fever.

In this work we associated with us in full confidence a commission appointed by the Mexican Government, consisting of Drs. Matienzo, del Rio, and Iglezias, all men of large clinical experience and ability, who agree to all the following statements in this case. There was also associated with us at this stage P. A. Surg. M. J. Rosenau, Director of the Hygienic Laboratory, who observed the case from September 5 to recovery, and who also agrees with the details as presented.

Experiment No. 1. A. G., aged 26 years, native of Jalapa; had been a resident of Teocelo, a mountain village, for many years and was an ironworker by trade. He had never visited the coast. This man had been a prisoner in one of the jails for the past twenty-two months and was brought from there to Vera Cruz with his full consent and permission and placed in a mosquito-proof room for observation and experimentation. He was examined physically and found to be free from organic lesions. The urinal examination was with negative results. An examination of fresh blood was made, which showed a few shadow blood corpuscles and blood platelets; the granulations of the polynuclear neutraphile were especially marked; Müller's blood dust was present; the red blood cells were of good color and form, and free from intracorpuscular bodies. The estimation of the blood elements is as follows:

Red blood cells			4,650,000
White blood cells			6,355
Hæmoglobin	per cent		80
(a) Lymphocytes	per cent	19.4	
(b) Large mononuclear	do	8.8	
(c) Polynuclear	do	67.4	
(d) Eosinophiles	do	4.4	
			100

The day following his arrival, September 4, at 9.30 a. m., two mosquitoes were permitted to feed on the left hand. These mosquitoes had previously been permitted to feed on a severe case of yellow fever on August 13, at 8 a. m., forty-one and one-half hours after the chill.



and had been fed on sugar and water to this date, twenty-two days and one and one-half hours. The mosquitoes were killed immediately after feeding and subsequently sectioned for histological examination.

The temperature, pulse, and general condition were taken regularly

at 8 a. m. and 4 p. m. without variation until September 7. On the afternoon of September 6 a small body slightly smaller than a red blood cell and containing four or five bodies that about filled the cell inside the membrane was discovered. These bodies moved slowly about one another, but as no more were found it did not justify further mention. On the morning of September 7 the patient wakened after a good night's sleep and had coffee and bread. At 11 a. m. breakfast was brought, but was not eaten with relish; at 11.30 a. m. a headache, especially frontal, developed, along with vague pains in the shoulders and knees. At 1.30 p. m. all pains were severe. There had been no chill, rigors, or nausea. The temperature was taken here and found to be 37.8, pulse 98. Counting from the beginning of the headache, it was seventy-four hours since the infection by the mosquito on September 4. The pulse and temperature went up rapidly. Some vomiting occurred after taking medicine in the evening and the following morning. The gums became congested, the eyes bright and suffused, and the case generally assumed the type of severe infection.

The blood was examined at this stage, but there was slight deviation from that previously reported. The character of the blood plate seemed to have undergone some modification. Instead of staining with a pale purple central zone with blue body, the central zone seemed to have broken up into highly fractile, small points, that stained beautifully with eosin and Goldhorn's methylene blue. For some time this excited considerable attention, but later it was found that normal blood of certain individuals gave reactions that closely simulated the above description.

On September 8 mosquitoes were contaminated by permitting them to feed on the hands and wrists. These mosquitoes were subsequently killed on different dates and examined histologically. It was decided to examine the blood systematically at night at two-hour intervals. On the night of September 9 fresh specimens and smears were examined. The specimens showed nothing abnormal. On September 10 an examination of the blood was made, with the following results:

Red blood cells	5,217,500
White blood cells	2,666

A differential count was not made, but after examining several hundred white cells the entire absence of eosinophiles was noted. The patient grew gradually weaker; the jaundice and bleeding from the gums became marked; black vomit subvened; the urine contained more and more albumin until $5\frac{3}{4}$ grams per 1,000 c. c. were recorded in the Esbach albuminometer. The albumin then came down to 4 parts per 1,000 on September 19, the day that the temperature returned to normal. The next day all albumin had disappeared, but the urine contained bile pigments in considerable amounts, but gradually returned to the normal. Owing to the rather critical condition

of the patient, all further attempts at study were abandoned until his complete recovery and discharge at the end of September.

The albumin in the urine was more or less remarkable on account of its rapid disappearance at the end of the disease. Following is a tabular statement of the urine:

Date.	Cubic centimeters.	Parts albumin per 1,000.
September 12	1,200	0
13	1,200	1
14	1,000	1½
15	1,100	4
16	1,200	3½
17	800	4
18	700	5½
19	850	4
20	1,000	0

In order to make this picture complete some repetition from the chapter on the contaminated mosquito is necessary. The mosquitoes used in this experiment were examined and in the salivary glands were seen spores filled with sporozoites in various stages of maturation. The mosquitoes in the same jar that did not feed showed the presence in their salivary glands of the same bodies as described and illustrated. In the mosquitoes fed on the experimental case and subsequently sectioned and examined the conjugating bodies are plainly seen on the fifth day after contamination. The remaining stages to the sporozoite are obscure on account of the diet, sugar and water, which was used. We believe that had we continued an albuminous diet, as in cases 11, 21, and 36, all the phases in these mosquitoes would have been as clearly presented as in the illustration.

After the success of this first case it was agreed to continue the experiments on other lines. In this continuation three more individuals were used without adding anything new to our knowledge of the disease.

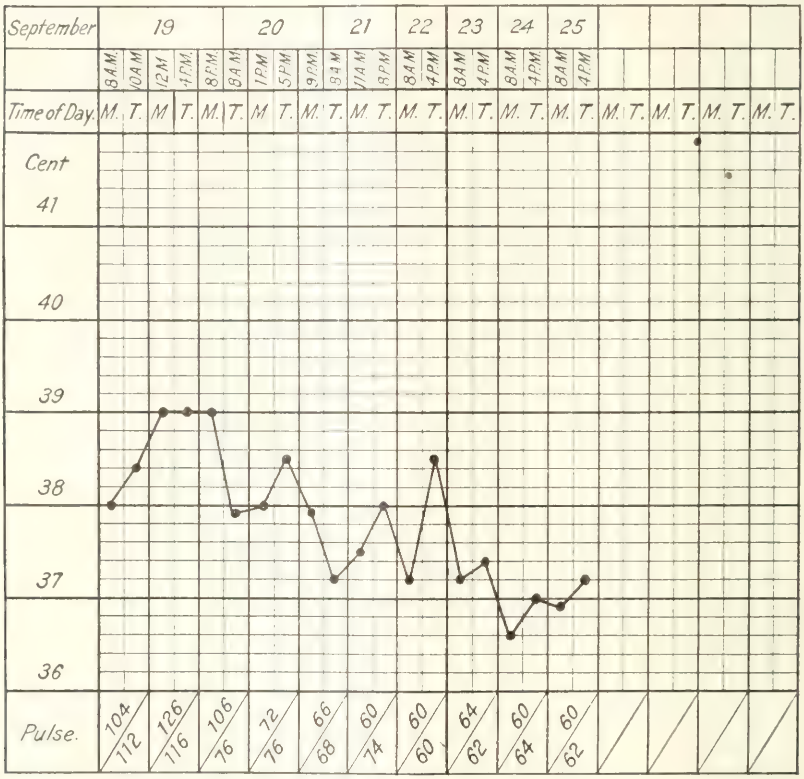
Experiment No. 2.—Benites; aged 27 years; a native of Jalapa; had lived there all his life with the exception of thirty days that had been spent at Vera Cruz some eight years ago; a shoemaker by trade; for the past nine months had been confined in one of the jails.

On September 9 at 9 p. m. this man was inoculated with blood serum from the vein of Experiment No. 1. The blood was drawn in sterile syringes and defibrinated. It was then diluted with two volumes of normal salt solution and filtered through a Berkefeld filter. 0.1 c. c. of this mixture was used for inoculation. The temperature of this man was taken every four hours for the next six days without variation from the normal. The experiment was then considered negative.

On the morning of September 19 he wakened with dull sensations in the back and legs. Shortly afterwards a headache and pain in the

knees and ankles developed. The temperature and pulse increased, and toward night a more or less typical case of yellow fever was diagnosed. This case was treated energetically from the beginning, and was mild after the first twenty-four hours. The urine was examined daily without detecting the presence of albumin. The case made a good recovery after five days of fever.

It is interesting to note that this case developed nine days twelve hours after the time of injection, and eleven days twenty-two and one-half hours from the time the first experiment was taken sick. From the history of the case that follows we agree that there must be some



loophole wherein a mosquito had gained access to the room, had become infected, and transmitted the infection to this man. It would seem more reasonable to believe this than that the period of incubation after the injection was nine days twelve hours.

Experiment No. 3.—A. C.; aged 21 years; native of San Antonio, State of Tlascala; lived in the neighborhood of Jalapa for the last nineteen years.

This man was inoculated with 1 c. c. of serum used to inoculate Experiment No. 2. Time of inoculation was 9 p. m. September 9. There was a slight febrile reaction on the following day, the tempera-

ture rising to 37.6, but returned to normal the following morning. From that time until his discharge on September 20 the man remained normal. He was transferred back to Jalapa and kept under observation several days with negative results.

Experiment No. 4.—This experiment was undertaken to discover whether infection could be conveyed by drinking water.

P. L.; native of Salaya; aged 22 years; had never been near the coast; for the last twenty-seven months had been in jail in the mountains. He arrived at Vera Cruz at noon on September 10 and was immediately placed in the mosquito-proof room. On September 11 four mosquitoes from the same lot used in Experiment No. 1 were crushed in drinking water and given the man to drink. The man was kept under observation until September 20 and then returned to the country, where he was still under observation. No reaction or deviation from the normal occurred during this period of observation.

8. SPECIES OF MOSQUITOES IN VERA CRUZ.

While most of the time and study of the working party was directed to the investigation of yellow fever, some attention was given to the determination of the varieties of mosquitoes, their distribution and habits, in the city of Vera Cruz and surroundings. Upon the arrival of the working party May 12, and for some time afterwards, but few mosquitoes were encountered, but as summer advanced the number of species increased until it had reached, at the conclusion of our work, a maximum of fifteen. This list, though large, is undoubtedly not complete, for, while the city is surrounded by low and swampy country, the almost complete lack of woods of any kind explains the absence of many sylvan mosquitoes and restricts these insects in their representation to the domestic and rural varieties alone. In the early part of our sojourn only four or five species could be identified and it was not until the latter part of June that other varietes were taken. The species determined by us appeared in the following order: *Culex pungens*, *Tæniorhynchus perturbans*, *Anopheles argyrotarsis*, *sub. sp. albipes*, *Culex confirmatus*, *Culex nigripes*, *Culex stimulans*, *Culex tæniorhynchus*, *Tæniorhynchus fasciolatus*, *Janthinosoma Lutzii*, *Anopheles maculipennis*, *Anopheles pseudopunctipennis*, *Culex sollicitans*, *Panoplites pseudotitillans*, and *Psorophora scintillans*.

Culex pungens, the first mosquito noted, is too well known to need any further description. Its life and habits are the same in Vera Cruz as elsewhere. Wherever found it is a gutter breeder, and the filthier the gutter and the more stagnant the water the greater its prolificacy. It may be stated here that owing to the daily and continual flushing of the gutters by the heavy rains this species was even less prevalent in Vera Cruz than in many other and more temperate regions. This species may be properly termed a domestic mosquito, preferring the

vicinity of human habitations to the more open swamp lands. It was found invariably associated with *Stegomyia*, breeding in the open barrels and water containers in the "patios" of nearly all the houses. The attacks of this insect are chiefly made after sundown and during the night, at which time we found them invading the houses in company with several species of swamp mosquitoes.

Tæniorhynchus perturbans, which commences its flight at about the same time as the preceding insect, is another well-known form in temperate regions. We found it in Vera Cruz only occasionally and in small numbers. Belonging originally to the genus Culex, it was placed on account of certain scale characteristics into a separate genus by Theobald. It is only upon a close examination that it may be distinguished from one of the most common and vicious swamp and night mosquitoes of this section, *Culex tæniorhynchus*.

Culex tæniorhynchus (fig. 35) in some respects, especially in the banding of the abdomen and legs, resembles, when freshly hatched, the female of the *Stegomyia fasciata*, but, of course, the absence of the very characteristic thoracic silver markings will readily identify it from the latter. Its usual breeding places are grassy water holes and canals in the city, and the large tract of swamp land, from which they are occasionally driven by strong winds into neighboring houses. In its tenacious manner of biting it resembles very much the common salt-water mosquito, *Culex sollicitans*, which seems to be rather uncommon in the vicinity of Vera Cruz, as only a few specimens were taken in the late summer.

Anopheles argyrotarsis, sub-sp. *albipes* (fig. 36), was one of the earliest mosquitoes found by us, though flying in small numbers. This small but handsome insect increased rapidly toward the end of summer and early fall and became a veritable pest. Until then cases of malignant quotidian malarial fever were rare. This disease increased proportionately with the appearance of this insect. From all the information we could gather at the San Sebastian Hospital and United States consulate, it has always been well known in Vera Cruz that as malarial fever is in the ascendency, yellow fever diminishes. This is readily explained by the preponderance at the time of either one or the other of the two varieties of mosquitoes that disseminate these diseases. During the latter part of July and especially during August and September the increase of *Anopheles* was very marked.

The *Anopheles argyrotarsis* was reinforced by the appearance of the well-known *Anopheles maculipennis* (fig. 37) and another, but evidently rarer species, *Anopheles pseudopunctipennis*. Suspected breeding places in the immediate outskirts of the city, which earlier in the season had no larvae or pupae, now contained thousands, and malarial fever cases increased with astonishing rapidity. *Anopheles argyrotarsis* is, as already stated, a rather small mosquito, not nearly as large

as the *maculipennis* or *pseudopunctipennis*. At first sight, especially when resting in the characteristic *Anopheles* position, it appears nearly black. Closer inspection shows the wing to be of an ochraceous tint, with a number of very dark brown spots, which on the anterior margin or costa are four in number and especially large. The thorax is a dark mouse gray, with three darker longitudinal lines, a median and two lateral lines commencing with a dark-brown spot; the median lines terminating in a black triangular patch. The abdomen is a very dark gray, bearing on each segment, excepting the last two, a triangular spot composed of yellowish-gray scales and on the sides a bunch of dark-brown scales standing at nearly right angles with the body, giving it a somewhat rough appearance. The occipital region is covered with and the joints of the long palpi are ringed with silvery-white scales. The legs are banded white apically. The tarsi of the middle legs are uniformly brown, but nearly all five joints of the hind tarsi are pure silver white, excepting the last one, which carries basally a dark, nearly black ring.

Anopheles pseudopunctipennis, n. sp., Theobald (fig. 38), is one of the handsomest species of this genus. In size and wing marking it resembles closely the *Anopheles punctipennis*. In fact, it might be mistaken for it were it not for the half black and white scaling of the posterior vein of the wings and also the white rings and dip of the palpi. The insect first made its appearance in the city toward the end of August, but we can not say whether it is a numerous species or not; but that it apparently understands the mission of tormenting the human race seems to be sufficiently attested by the fact that the specimens in our possession were captured inside of the mosquito bars.

Anopheles maculipennis (fig. 37) is by this time too widely known to need any further description. In Vera Cruz and surroundings it became fairly common in August, but did not occur in such large numbers as *Anopheles argyrotarsis*.

Whether it has any relation to the malignant quotidian malarial fever or not we are at present not prepared to say. Its absence during the earlier part of our sojourn and the contemporaneous existence of *A. albipes* and this variety of infection would suggest, in a measure, the negative. With its appearance in appreciable numbers in the month of August we also found a larger number of cases of tertian malarial fever, not only among the hospital cases but among the citizens of the city. This part of the subject could not be pursued by us, but it is certainly one of the most important for the improvement of the hygienic conditions of Vera Cruz and other parts where these two mosquitoes exist side by side.

The remaining mosquitoes are of little consequence, as, for instance, *Culex stimulans*, which were observed by us in a few instances. It does not seem to be a very numerous species anywhere, and it was

somewhat a surprise to us to find it so far south as Vera Cruz. Owing to the small numbers in which it was observed no particular attention was given to it.

The same remarks may also apply to *Culex confirmatus* Arribalzaga (fig. 39), for while several specimens were taken by us in the city, it can not be considered as an insect of importance or as one having any relation to hygiene. Since its original description by Arribalzaga the distribution of this insect has been found to be even greater than given by Theobald. It is found not only in the United States, but this locality has now been added to its habitat. It is a handsome insect, of medium size, conspicuous in appearance on account of the large patch of silver-white scales on the thorax. Its eggs and the manner of depositing them are somewhat similar to the *Stegomyia fasciata*.

During the month of June our laboratory was invaded nightly by a mosquito which heretofore had scarcely been regarded as a southern species, but which, according to our experience in Vera Cruz, it no doubt is. This mosquito, *Culex nigripes*, or, better, *Culex impiger*, is, wherever it does occur, a veritable nuisance. In size it is small, but a stoutly built insect. Its flight was an exceedingly short one, and was over after two or three weeks' duration.

About the middle of August one of the sanitary officers brought a number of very large larvae and pupae to the laboratory, which had been obtained by him from a small pond just outside of the city limits. In the course of two or three days the adults emerged, which, compared with the size of the larvae, were very small. In fact, we believed at first that the larvae were those of *Psorophora*. They proved to be *Taeniorhynchus fasciolatus* (fig. 40). Unfortunately, owing to the other pressing work, we failed to obtain any illustrations or measures of the larvae and pupae, which at this time are still undescribed. The single insect from which the illustration was made we secured much earlier in the season, June 25th. The insect is dark colored and large, the thorax is very dark-brown, somewhat lighter in the middle and carries on its side a patch of white scales. The abdomen is nearly black, with six silver-white spots just above the pleural membrane. The chief characteristic of this species is the pure white ring or band on the apical end of each femur. Theobald only reported this mosquito for Brazil and Argentina.

Another species of mosquitoes which we determined and which only thus far has been recorded in the lower Amazon and Rio de Janeiro is *Janthinosoma Lutzii* (fig. 41), described by Theobald as a new species. It is a large and very handsome insect adorned with brilliant metallic blue and purple scales on the abdomen and legs, and golden-yellow scales on the thorax. Its flight in the city was very short, but in the swamps outside of the city limits it was a veritable scourge. Its bite is very severe and irritating for a long time. It entered the shipping

in the harbor, as we determined by examination of the vessels in port, where among a variety of mosquitoes found in the quarters and holds *Janthinosoma Lutzii* was one of the most numerous.

Psorophora scintillans Walker (fig. 42) is another species whose range has been extended according to our observations. Theobald or rather Walker, records it only for Para. How Theobald comes to describe the insect with the proboscis curved as in *Megarhinus* it is impossible to understand. All of our specimens had a straight proboscis during life and retained it after death.

The fourteenth species, *Panoplites pseudotitillans* Theobald (fig. 43) was noted by us at the latter part of our stay. It is a small brown and yellow, but dark looking insect and no doubt is a swamp mosquito.

The general characteristics of *Panoplites* are in this species exceedingly well represented by the densely sealed wing veins; the scales themselves are broad and asymmetrical. In the city we did not find the insect in numbers, but they were reported to us as being exceedingly vicious in the swamps, and especially along the seashore. Since *Panoplites* has been shown to act as the host to *Filaria Bancrofti*, some attention should be given to the life history of this mosquito. Filariasis, however, is said to be rare in or about Vera Cruz, only one reported case being known to the members of the working party. No examination of the case was made to verify the disease.

9. THE TRANSFERENCE OF MOSQUITOES BY SHIPS.

This subject was not studied in detail on account of lack of facilities, but sufficient information was gathered to make it worth mentioning. The only time we found mosquitoes actually breeding on board a vessel was in the instance of the American schooner *John H. Crandon*. This schooner had arrived from Mobile, Ala., with a cargo of lumber twenty days previous to our examination. Water and provisions had been taken on at Mobile. The examination was made on account of a case of yellow fever occurring among the crew. The wooden tanks were examined and found to contain larvae of *Stegomyia* by thousands. The iron water tanks were examined, but owing to their depth and small openings none were found. Iron water tanks, as a rule, are more securely covered and are given more attention than the wooden containers. The two or three barrels, placed in front of the galley and forecastle, are more frequently infested and in every instance preference was given them in the examination. The box of carpenter's grindstone offers another favorite place for the breeding of this insect. An examination of the cabins was made to determine the presence or absence of adult insects. In the room from which the man with yellow fever was taken, the insects were found in abundance, both normal and blood fed, resting in the shady corners on dark objects hanging around the room. Considerable effort is necessary to detect mos-

quitoes when they are few in number; each cabin should be gone over systematically, passing the hands in the corners and gently shaking the hanging articles until every object above the floor has been gently disturbed. The forecastle and galley of this schooner, immediately behind the breeding barrels, harbored the insect in abundance.

The steamship *William Cliff* is of interest in connection with the transference of mosquitoes by vessels as well as furnishing data relative to the period of incubation and spread of malaria among a crew where all outside influences were removed.

This vessel sailed from Liverpool, England, on May 29, 1902, with the following itinerary:

Arrived.	Port.	Sailed.
June 11	St. Thomas	June 12
June 15	Colon	June 16
June 19	Kingston	June 20
June 25	Tampico	June 26
June 27	Vera Cruz	July 1

Until arrival in Tampico no sickness had occurred on board; while leaving that port one man was taken sick with a vague chill, and illy defined pains. Two days later, June 28, four of the crew were taken sick with pronounced chills, pains in the head, extremities, and back, and high fever. The following day, June 29, four more including the captain, were taken sick in like manner; on June 30, two more were taken sick.

On June 29, the blood of two of the cases was examined for the plasmodium malaria with negative results. Their absence was accounted for by the large doses of quinine administered prior to the examination. On July 1, two new cases were examined; in one the tertian parasite was obtained, the other was negative.

The disease clinically was of the tertian type, about half-intermittent, the balance remittant. All, with one exception, responded readily to quinine. The exception resisted the quinine for three days. An examination of the ship was made to determine the genera and species of mosquitoes on board. Of these there were three genera and four species in abundance, as follows:

Anopheles argyrotarsis,
Culex fatigans,
Culex teniorhynchus,
Janthinosoma Lutzii.

All the varieties mentioned were found in the forecastle and after wheelhouse, the crew's quarters being aft instead of forward. The cabins failed to show the presence of mosquitoes on the examination of June 30, nor were any noted previous to that time.

Anopheles argyrotarsis were present in Vera Cruz, though in very

small numbers; so scarce, that up to that time only one specimen had been taken. This mosquito is also native to Tampico and Colon. According to the history of the crew, which on such subjects is very unreliable, they stated that while at Tampico they had not been molested by mosquitoes, while at Colon all hands suffered severely from bites. The character of the fever is extremely suggestive of a Colon infection, the tertian type being comparatively rare, both in Tampico and Vera Cruz. Another point that makes Colon infection seem positive is the period of incubation that invariably takes place after primary malarial infection. This period has been variously stated to be from one day to several weeks. Our own observations (Parker—report to Surgeon-General, October, 1901) on this point are limited to a single observation that included three crews, one of a steamship and two of schooners at Jacksonville, Fla., during the fall of 1901. In this instance the three vessels arrived at Jacksonville from northern ports at about the same time, and lay at a badly infected part of the town. Cases of malaria appeared simultaneously on all three boats fourteen days after arrival.

Dr. D. D. le Favre (Roussky Vratch, October 19, 1902), in an experiment on himself, showed that the actual period of incubation of malaria in one instance was twelve days.

Those long periods of incubation in the malignant tertian and quotidian undoubtedly account for many infections that have been classed as being yellow fever.

It is a well-known fact, which one of us (Parker) has experienced, that steamers lying in Habana Harbor become badly infested with mosquitoes, which insects remain on board the steamship during the voyage to Progreso and thence to Vera Cruz, appearing at night in the cabins in considerable numbers.

It will be seen, then, that there is considerable evidence to support the assertion that these insects are constantly being transferred from one locality to another through the medium of ocean commerce. In this distribution the iron ship, on account of its concealed water tanks, acts in the nature of a mechanical host, while wooden vessels, on account of the loosely covered deck containers, not only act as a mechanical host, but under favorable conditions, as in calm or gentle weather, or lying at anchor, furnish the proper medium for the propagation especially of domestic species.

Passed Assistant Surgeon Grubbs, U. S. Public Health and Marine-Hospital Service, has collected data at the Gulf Quarantine Station relative to the transference of mosquitoes by vessels. In a total of 82 vessels inspected to determine the presence of these insects, he found *Stegomyia fasciata* in the adult stage on three wooden vessels, all from Vera Cruz. One of the vessels was the *John H. Crandon* mentioned above.

10. MOSQUITO TECHNIQUE.

The stimulus and much of the credit of all this work must be given to Ross and Manson, because of their association of the extra-corporeal cycle of the malarial hæmosporidia with the various species of *Anopheles*. Later the stage of maturation of the *filaria embryo* was studied and proved in connection with the epidemiology of that disease. In 1900, Graham, working in Syria, associates a cycle in the various species of *Culices*, in the transference of the contagion of dengue, and while this work has not been confirmed, it must be given a certain amount of credence. The work of Reed and Carrol in connection with the subject of yellow fever has been reviewed.

In order to be able to appreciate the changes that take place in the body of the mosquito after contamination with disease, it is essential that the normal anatomy and histology should be clearly comprehended. This can only be accomplished by actual work with the mosquito, both in the fresh state and in sections. In the following section it is our aim to give only the essentials of this interesting work. There are certain parts that we feel are not yet comprehended in sufficient detail to make more than casual mention.

All mosquitoes used in experimental work must be, as ours were, carried through the larval and pupa stages in the laboratory. The larva should be collected with care, as there are many variations in size; the large ones serve the purpose better and are much more readily handled than the smaller ones. The larva should be placed in clean jars with wide mouths; a sleeve of cheese cloth 18 inches long surrounding the neck serves to prevent their escape and makes the adult insect accessible. A sufficient quantity of water, either from the breeding place, which necessarily contains abundant food stuff, or fresh water to which has been added a small quantity of Spirogyra for food, should be placed in the jar. A subdued light is more favorable for their development.

To secure mosquitoes for examination and contamination, they should be taken out singly in a short test tube, selecting the largest and most active insects. Those for immediate examination should be killed with fumes of tobacco, as that poison relaxes all the structures. Chloroform and ether strongly contract the tissues and seem to make them brittle; for immediate or fresh examination they should be transferred as soon as overcome to a neutral fluid, such as normal salt solution or glycerin, or dissected dry.

While the dissection of the fresh insect possesses advantages in quickness, in gross anatomy, and for determining the presence of mature zygotes in the stomach wall of the species of Anopheles, it is altogether unsuited for histological and accurate work. The salivary glands and stomach can sometimes be removed en masse by gentle

traction on the head with a flat needle. With a little practice, the salivary glands may be examined *in situ* by carefully approaching those structures through the dorsal tissues.

Paraffin or celloidin embedding is more complicated than the above, but possesses greater, and when cut and mounted serially, absolute accuracy.

The steps in the process may be briefly described as follows:

Killing. Two methods were employed: First, the mosquito was taken from the jar or cage in a small test tube, stunned with tobacco smoke, and then placed in the fixative; second, instead of using tobacco smoke, the test tube was inverted over a small bottle of fixative; the mosquito soon fell into the fluid.

Fixing.—We have separately and collectively used all the fixatives used in ordinary and special histological work. There is no doubt that bichloride of mercury, osmic acid, and the salts of chromium, in their various combinations, give the same excellent results as fixatives in mosquito work as in histological work. The mosquito, however, can not be manipulated after fixation as ordinary histological specimens, on account of the liability to break certain parts of the chitin. Even with special effort to remove every trace of the fixative, a certain amount always precipitates as an oxide, which prohibits their use in this connection. Cut sections filled with or containing pigment in parts is a source of error which must be guarded against.

In order to obviate the disadvantage mentioned, absolute alcohol was selected, and proved so satisfactory that it was used throughout the work. The mosquito was permitted to fly or fall directly into the alcohol and remain there one or two hours; it was then transferred to a watch glass, the legs pulled off with forceps, the wings carefully cut near their attachments, and then transferred to another change of absolute alcohol for two hours to secure *dehydration.*

Clearing.—After the second change of absolute alcohol the insect is transferred to xylol, where it remains until clear, usually half an hour. It is then transferred to another change of xylol, and placed in the warm chamber of the paraffin bath. Here, from time to time, a few drops of liquid paraffin are added until the xylol is well saturated. It is then transferred to soft paraffin for one to two hours, then to paraffin of 52 melting point, and after one hour is ready for embedding.

Embedding.—The same general features are used in the embedding of mosquitoes as in histological work. It is better, however, to embed on small blocks of wood that have a coating of hard paraffin about 2 mm. thick on the embedding surface. This is inclosed in a paper box. The box is filled with melted paraffin, the mosquito is taken by the proboscis and quickly transferred to the box, placed in position, and then put on ice for rapid congelation. When the block is

orientated the mosquito will be found in perfectly translucent paraffin, so necessary for good serial cutting.

Orientation.—Care should be taken not to approach too closely to the mosquito, as our experience was that the chitinous structure cuts much better if supported by a generous portion of paraffin. The orientated block should be oblong, and for the serial sections that are to follow should have accurately cut parallel sides.

Sectioning.—We were unfortunate in the selection of a microtome for this most important work, having taken one of the sliding microtomes after the model of Schanze. The Minot rotary microtome is about the only model that gives good results, though the Cambridge rocking microtome is a fair substitute. Two things are essential for success in paraffin sectioning in the Tropics: the block and instrument should be chilled and the sections cut in the early morning when the temperature may be several degrees lower. The sections should be cut serially and immediately placed in cold water to permit them to flatten before mounting. The knife should be examined frequently during the sectioning, as the chitin sometimes turns the edge, thus ruining the sections that follow.

The thickness of the section is a matter of considerable importance; it depends upon the nature of the work, whether for histological study or for study of developing sporozoa. In the first instance, a uniform thickness of 20 micra is about as thin as can be depended upon; in the latter an average of 15 micra. Sections of 10 micra or less show too little structure to be of service and possess no advantage for any phase of the work.

Sagittal sections are better adapted for manipulation and examination than other sections. The anatomic planes can also be more easily followed.

Mounting.—Two or three rows of sections, each about 45 mm. in length, should be floated on a slide and the water drained off. The specimen should then be covered with rice paper, moistened with 80 per cent alcohol, and then flattened with blotting paper under careful but considerable pressure. The slides should then be set aside, and protected from dust until thoroughly dried. Unless this latter mechanical dehydration is perfect, the section is apt to wash off in the subsequent treatment. If dry, the specimen will stand the same amount of manipulation as though a mounting medium had been used.

The use of albumen, acacia, or starch fixatives is condemned as being unclean and unnecessary.

Removing the embedding material.—This is accomplished with xylol in the same manner as in histological work.

Staining.—This most important part of the work is still in its infancy. It can not be said that there is a single or combination of stains that give results that are perfectly satisfactory. We have tried at various

times haemotoxylon, either alone or in combination with eosin, Romanowsky's with Jenner's modification, and the various preparations of methylene blue and thionin, with the same results—partial success.

The stains that gave us the best results, and with which we believe the whole future work will depend upon, were the iron haemotoxylin, after the method of Heidenhein, and a saturated solution of Bismarck brown in hot 60 per cent alcohol. The results in either instance, when properly applied, will be a precise nuclear with a faint ground stain, or, with further differentiation, a purely nuclear stain of great brilliancy. In choosing between these two stains it should be remembered that the haemotoxylin is specially adapted to the staining of some phases of protozoa, while the latter selects the protozoa secondary to the histology of the insect.

Dehydration.—Dehydration is secured by passing through the alcohols, and *clearing* with xylol. The specimen should be *mounted* in xylol balsam.

Cover glasses, 22 by 50 mm. should be used to prevent overlapping of sections or cover glasses, which invariably results from the use of smaller sizes.

Celloidin embedding.—This method finds much favor with English and continental observers and has some few adherents in this country. It was given a trial, but on account of our inability to do serial work was discontinued. We might say that it has less to recommend it in mosquito work than in finer histological work, where it has been generally abandoned. The following is the technique used for embedding:

1. Dehydration through alcohols.
2. Transfer mosquito from absolute alcohol to a mixture of absolute alcohol and ether, equal parts.
3. Transfer to thin celloidin for forty-eight hours.
4. Transfer to thick celloidin for forty-eight hours.
5. Transfer to paper mold on cork, filled with thick celloidin.
6. Transfer to 70 per cent alcohol for three days.
7. Cut sections with knife flooded with 70 per cent alcohol.
8. Stain.
9. Dehydrate.
10. Clear.
11. Mount.

MOSQUITO-PROOF ROOMS.

It is necessary in all infected centers to have quarters provided for the reception of yellow-fever cases. It will not be found necessary or advisable to go beyond the city limits to procure these quarters, as a room or ward can be made mosquito proof and thus save the patient the needless transportation which experience shows enhances the mortality of every epidemic.

A room may be hastily provided by taking a good quality of cheese or other cloth, having from 20 to 30 meshes to the inch, and placing

it over all windows and doors. The entrance should be further protected by double doors, sufficiently separated so that only one can be opened at a time. Permanent quarters should be protected by brass-wire screens, about 20 meshes to the inch, with a vestibule to preclude the possibility of mosquitoes entering. All drainage connections from this ward should be well trapped or covered with wire screen. It is safer, however, to do away with this source of contamination by directly disinfecting the excreta and transferring it in buckets to the sewer or incinerator.

Preparatory to occupying a room designed for the reception of patients having this communicable disease, all insects should be killed by one of the good insecticides. Too much dependence should not be placed in pyrethrum and tobacco, as experience has taught that a concentrated atmosphere is necessary to produce death, and too many loopholes are offered for escape. All cracks in the floor, narrow ledges over the windows and doors, should be examined, and the room should be carefully dusted and swept while the effects of the drug are still in it. A much more effective method is by sulphur dioxide. This agent, in small proportion, is rapidly destructive to all insect life occupying bedrooms and quarters generally. It is readily prepared by burning flowers or roll sulphur in the room. One pound of sulphur to every 1,000 cubic feet of space with two hours' exposure may be considered safe. Should the house or cabin contain furnishings of value, this sulphur disinfection can be accomplished without damage to these articles, providing attention is given to the production of a dry gas. The liquid sulphur dioxide (2 pounds per 1,000 cubic feet) can be used for this purpose on a dry day without injuring fabrics of any kind. Should the air contain moisture, however, the SO_2 combines with the H_2O, forming H_2SO_3, which is an energetic bleaching agent.

Toward the end of the epidemic in Vera Cruz Dr. del Rio provided a portable room, 8 feet long and 6 feet wide, covered with wire screen. This can be taken apart, placed in a room, the patient placed therein, and for temporary use this is a good means of limiting the infection for the time being.

It may be mentioned here that scientific experiments on either malaria or yellow fever lose considerably in value when conducted in accurately prepared quarters in cities where either of these diseases are endemic. There are so many sources of error that their elimination is to a certain extent relative. It is, however, justifiable after due precaution to do this work for positive or negative data; the gradations between the two, that so frequently appear in work of this character, should have every source of error eliminated by conducting the experiment in regions enjoying a well-established immunity to these diseases.

○

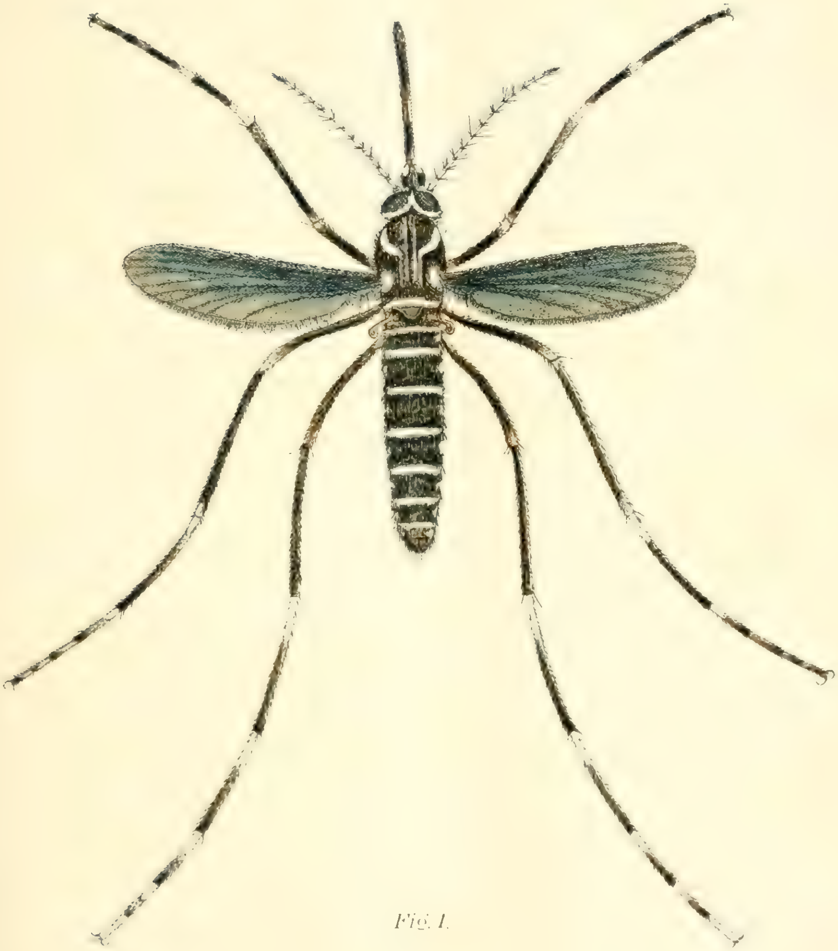


Fig. 1.

STEGOMYIA FASCIATA FABR. FEMALE.

Fig 2.

STEGOMYIA FASCIATA FABR. MALE

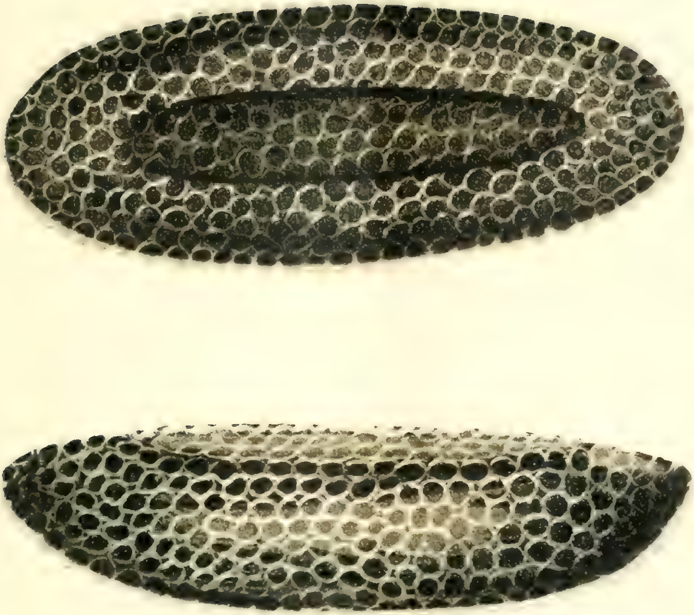


Fig. 3.

EGGS OF STEGOMYIA FASCIATA, UPPER AND SIDE VIEW.

AMPLIFICATION 150

Fig. 1.

Mature Larva of Stegomyia fasciata.
Respiratory siphon of Culex to the right.

Fig. 5.

PUPA OF STEGOMYIA FASCIATA FABR.

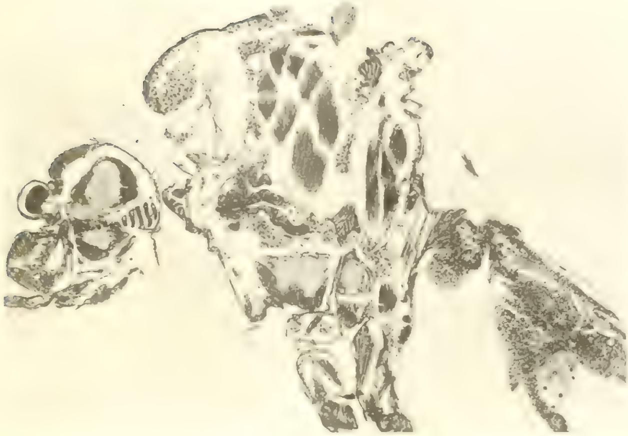


FIG. [illegible]

SAGGITAL SECTION OF FRESH UNCONTAMINATED STEGOMYIA FASCIATA SHOWING RELATION OF SALIVARY GLANDS.

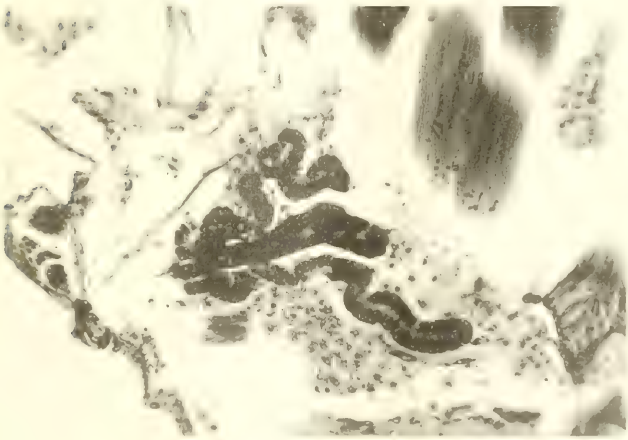


FIG. 7.

SAME AS FIG. 6, ENLARGED, SHOWING SALIVARY TUBULES IMBEDDED IN LOOSE CONNECTIVE TISSUE; BEHIND THE TUBULES, THE ESOPHAGEAL DIVERTICULUM.

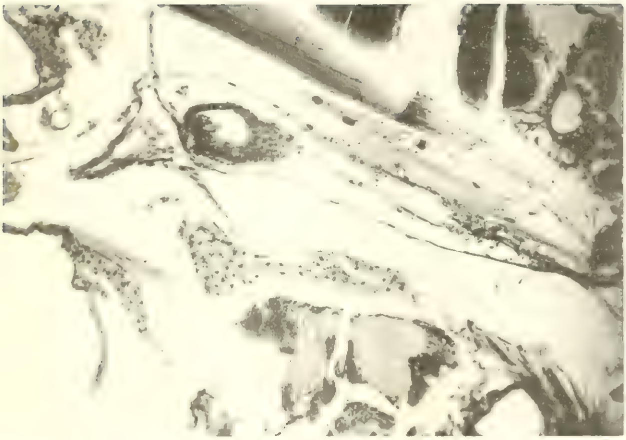


FIG. 8.

[SA]ME MOSQUITO AS FIGS. 6, 7, SHOWING MEDIAN PLANE OF MOSQUITO, A. ESOPHAGUS; B. HOMOLOGUE OF PROVENTRICULUS; C. UPPER AND LOWER BORDER OF ESOPHAGEAL DIVERTICULUM, D. THORACIC GANGLION.

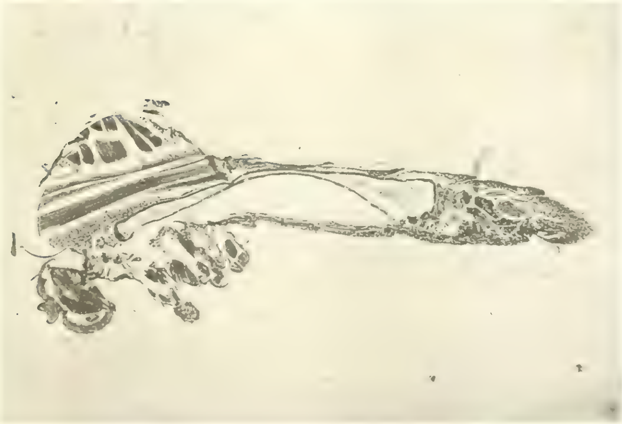


FIG. 9.

SHOWING THE WHOLE MID-GUT (THE STOMACH) THE ANTERIOR CONSTRICTED AND POSTERIOR DILATED PORTIONS; BELOW, THE ESOPHAGEAL DIVERTICULUM.

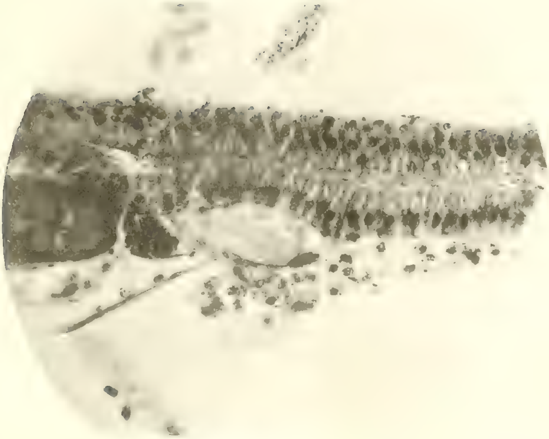


FIG. 10.

EPITHELIUM OF MID-GUT, THE TWO LAYERS IN CONTACT.

FIG. 11.

NORMAL *Stegomyia Fasciata* SHOWING ANATOMY OF HIND-GUT: A, ESOPHAGEAL DIVERTICULUM. B, MID-GUT. C, PYLORIC DILATATION. D, COLON DILATATION. E, RECTAL DILATATION AND GLANDS.

FIG. 12.

NORMAL STEGOMYIA FASCIATA SHOWING RECTUM AND RECTAL GLANDS.

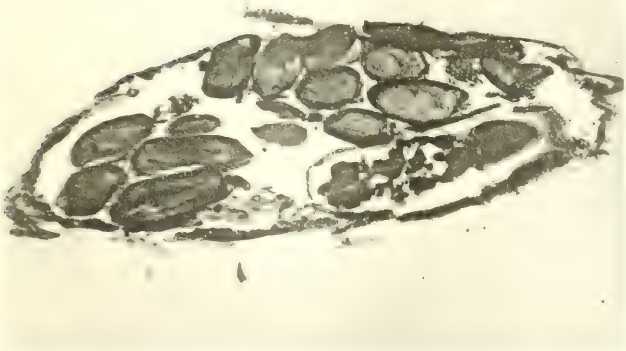


FIG. 13.

MATURE OVA OF NORMAL BLOOD

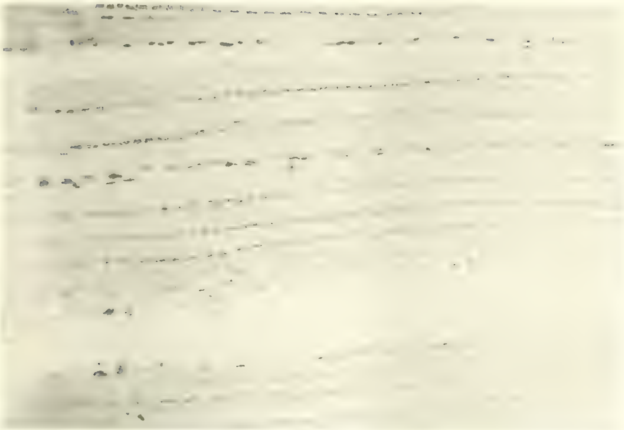


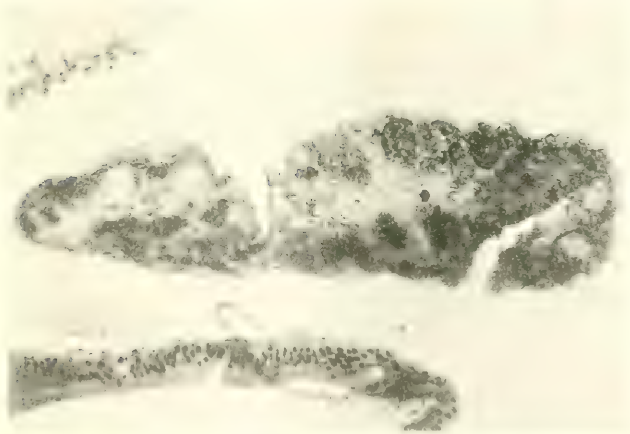
FIG. 14.

NORMAL MUSCLE PLATES OF THORAX OF *Stegomyia Fasciata.*

FUSIFORM SHAPE PROTOZOA, THE MYXOCOCCIDIUM STEGOMYIAE,

IN STOMACH AND [illegible] DIVERTICULUM.

…OID MASS.



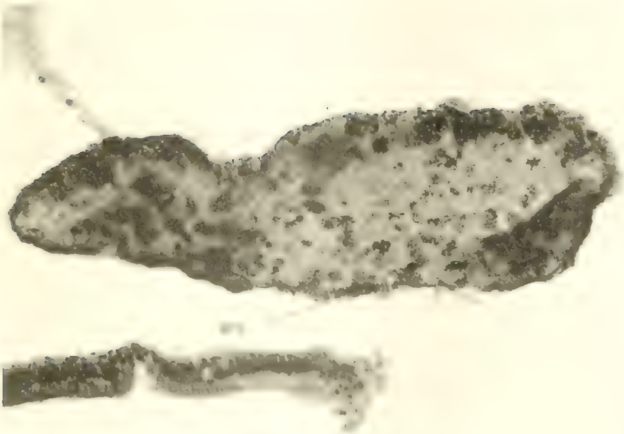
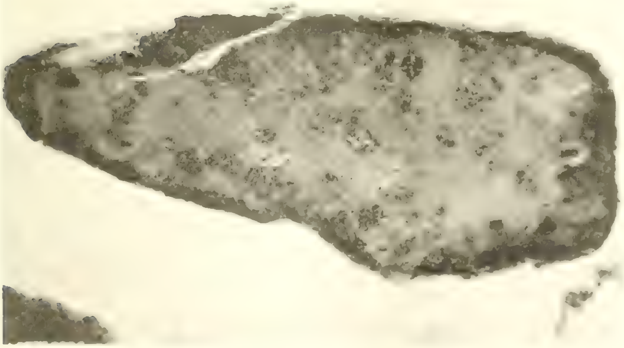


FIG. 18.

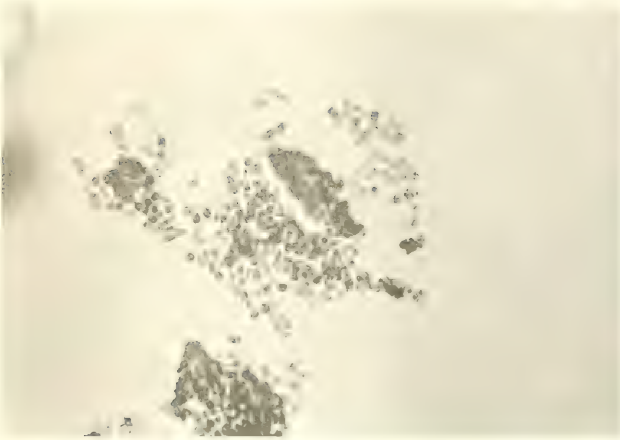
SAME MOSQUITO AS FIG. 17. SHOWING INTERIOR OF ALBUMINOID MASS.

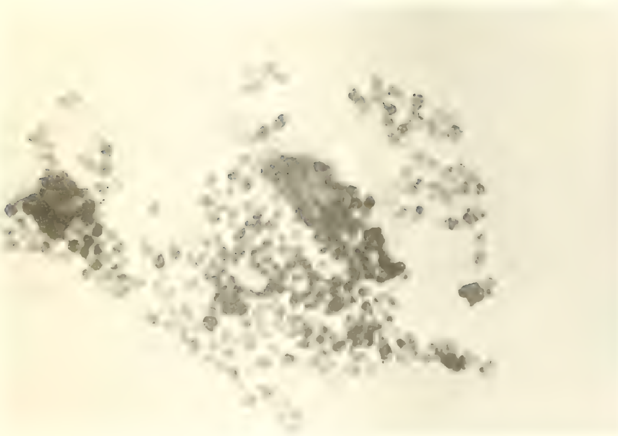


SAME SECTION AS FIGS. 17, 18 SHOWING CENT[illegible]NOID MASS.

FIG. 23.

[illegible]GEMENT OF SPOROBLASTS?





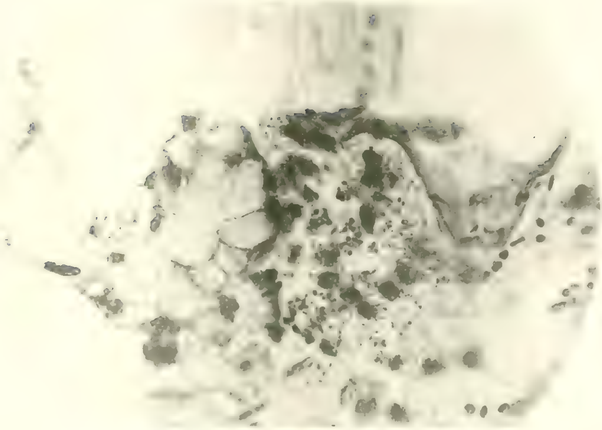


FIG. 27.

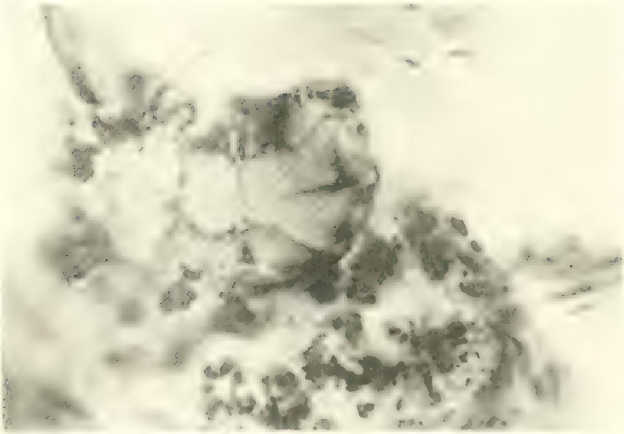


FIG. 28.

SHOWING THE "SPOROBLASTS" DISSEMINATED [illegible]

TISSUE SUPPORTING THE SALIVARY GLAND.

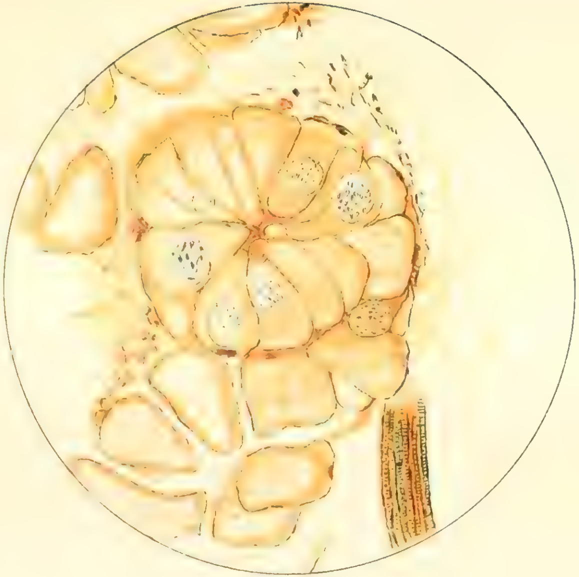
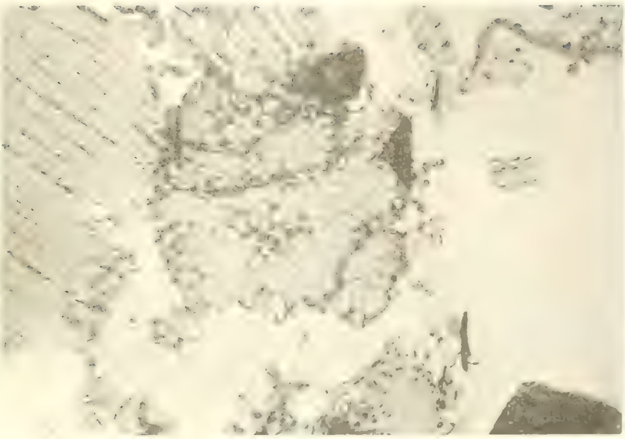


Fig. 29.

Fig. 30

Fig. 29. 30 SHOWING THE "SPORE?" CONTAINING THE SPOROZOITE? IN THE SALVARY GLAND CELLS

SHOW DEGENERATION OF OVA IN CONTAMINATED MOSQUITOES



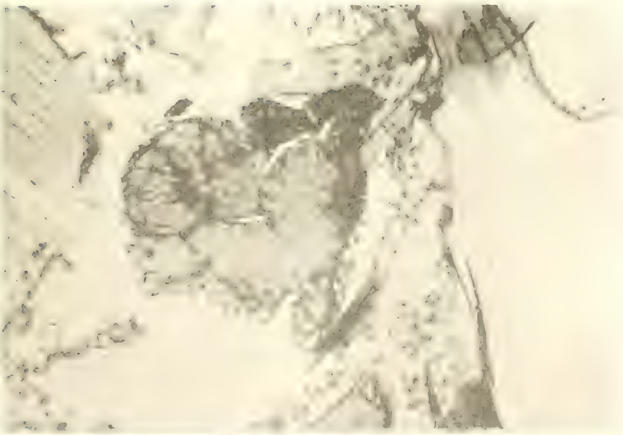


Fig. 35.

CULEX TAENIORHYNCHUS WIEDEM. FEMALE

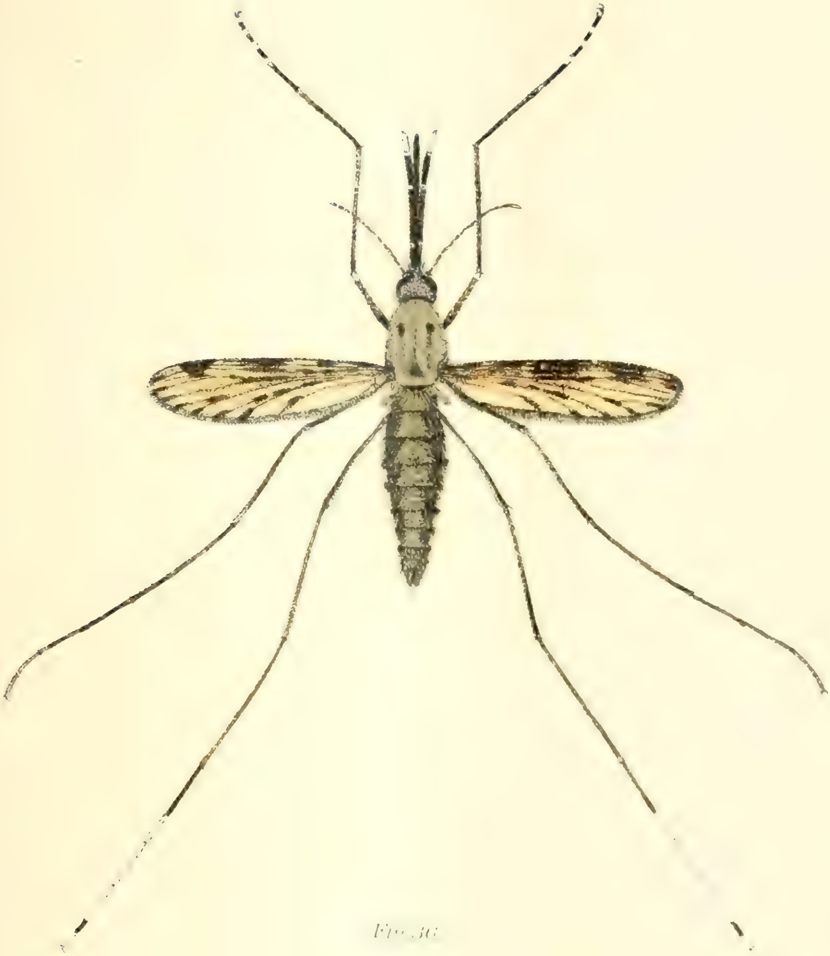


Fig. 36.

An[illegible]tarsis Desv.

Subspecies: albipes Theo.

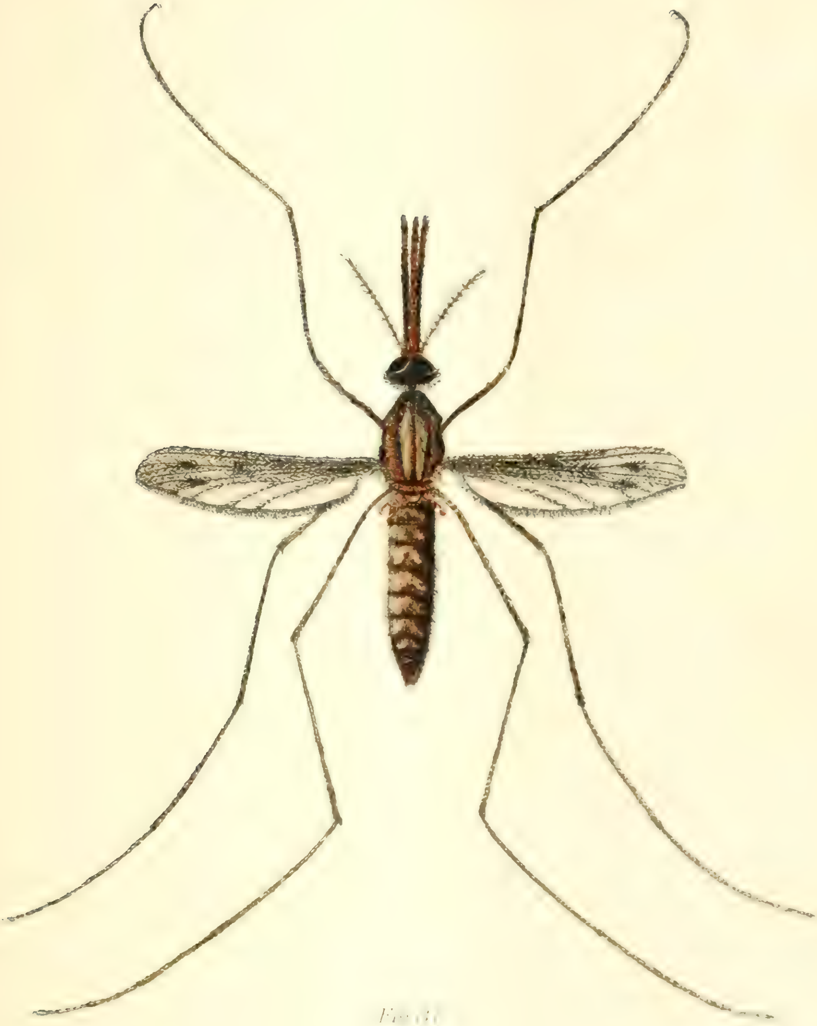


Fig. [illegible]

ANOPHELES MACULIPENNIS MEIGEN

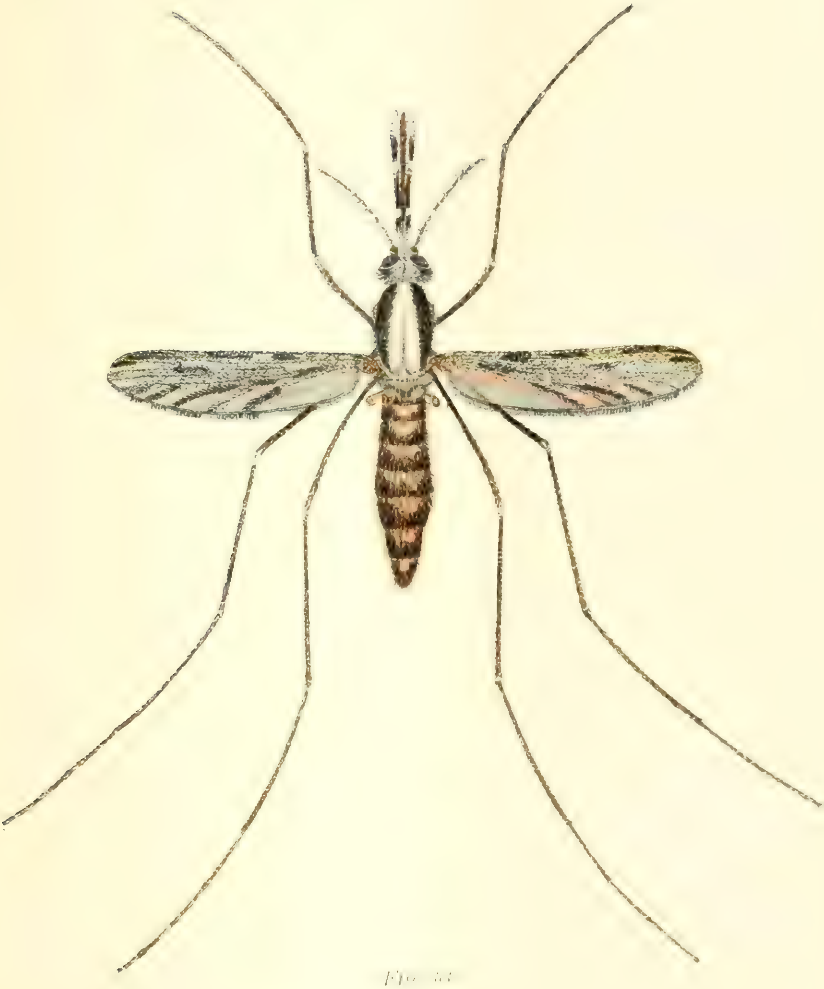


Fig. [illegible]

ANOPHELES PSEUDOPUNCTIPENNIS THEOBALD.

Fig. 38

[illegible] FIRMATUS

[illegible]

Fig. 10.

TAENIORHYNCHUS FASCIOLATUS ARRIBALZAGA

Fig. 41.

JANTHINOSOMA LUTZII ARRIBAL.

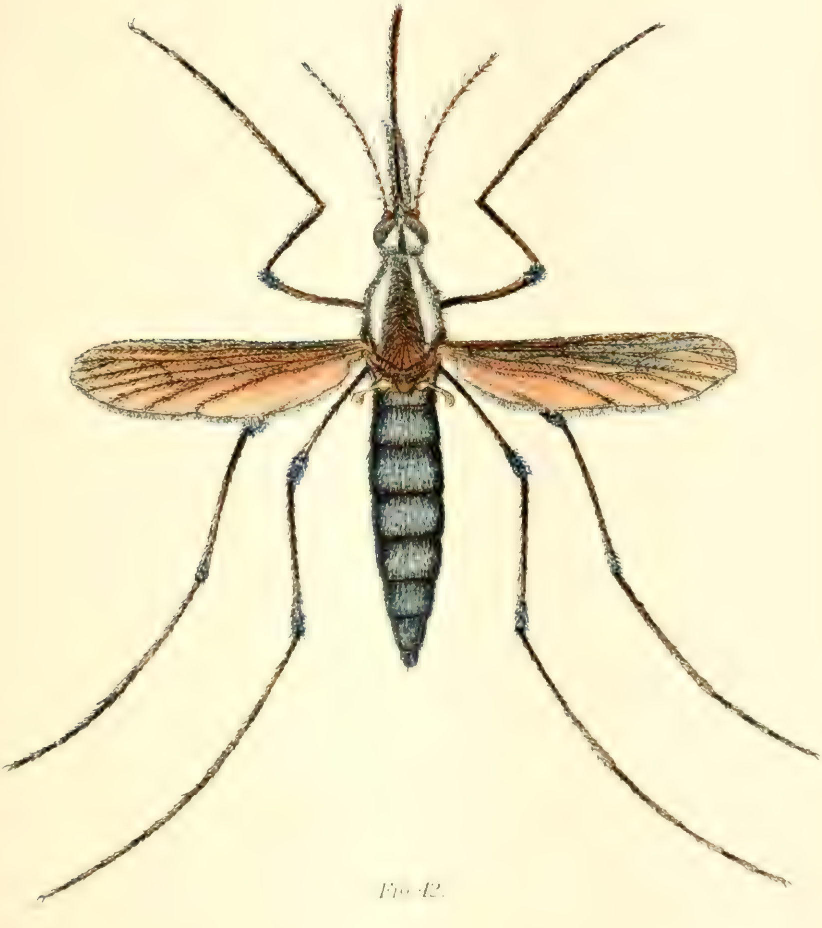


Fig. 42.

Psorophora scintillans Arribalzaga.

Fig. 43.

PANOPLITES PSEUDOTITILLANS THEOBALD.
WINGSCALES BELOW.

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